ANGIOTENSIN II CONTROL OF REGIONAL HAEMODYNAMICS IN RATS WITH AORTOCAVAL FISTULAE

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Angiotensin II control of regional haemodynamics in rats with aortocaval fistulae

by

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Abstract

Pathological conditions exist in which regional blood supply is reduced due to a decrease in arterial blood vessel lumen diameter. This may be accomplished by humoral or neurogenic vasoconstriction, or by arterial blood vessel hypertrophy. Adequate blood perfusion is important, and it is desirable to improve blood supply in these conditions. Rats with aortocaval fistulae (ACF) are models of heart failure in which regional blood supply is reduced and the renin-angiotensin-aldosterone system (RAAS) is activated. Attenuation of the RAAS was expected to cause a larger decrease in mean arterial pressure (MAP) and larger increases in regional blood flows and conductances (haemodynamics) in ACF rats than in sham-operated (SO) rats. Furthermore, AT_1 receptor antagonism by losartan was expected to cause larger effects on MAP and regional haemodynamics than AT_2 receptor antagonism by PD 123319.

The continuous influence of angiotensin II on regional haemodynamics was estimated by administering captopril, losartan, and PD 123319 to rats with and without ACF. Losartan caused a larger decrease in MAP and generally larger increases in regional haemodynamics in rats with ACF than in SO rats. In rats with ACF, losartan caused a larger decrease in MAP and larger increases in regional conductances than the decreases produced by PD 123319. In SO rats, the increase in MAP produced by PD 123319 was larger than the decrease produced by losartan. The increases in mesenteric and renal conductances produced by losartan were larger than the decreases produced by PD 123319. However, the decrease in iliac conductance produced by PD 123319 was larger than that produced by losartan.

The results of this study suggest (1) that the RAAS has a greater influence in controlling regional haemodynamics in rats with ACF than in SO rats and (2) that the RAAS is altered in rats with ACF to favor vasoconstriction, possibly as a mechanism of maintaining a normal mean arterial pressure despite a large decrease in total peripheral resistance. Finally, (3) AT_1 receptor antagonism is more effective than ACE inhibition at increasing regional blood flow in rats with ACF. This is important, given the goal of increasing regional blood supply in these rats, and may be true of other pathological conditions in which tissue ischaemia results from systemic vasoconstriction and decreased systemic blood flow.

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List of Abbreviations and Symbols

ABF: Aortic blood flow

AC: Aortic conductance

ACE: Angiotensin-converting enzyme

ACE2: Angiotensin-converting enzyme 2

ACF: Aortocaval fistula

Ang 1-7: Angiotensin 1-7

Ang 1-9: Angiotensin 1-9

Ang I: Angiotensin I

Ang II: Angiotensin II

Ang III: Angiotensin III

Ang IV: Angiotensin IV

AT_1: Angiotensin II receptor type 1

AT_2: Angiotensin II receptor type 2

AT_4: Angiotensin IV receptor

COX-1: Cyclooxygenase-1

COX-2: Cyclooxygenase-2

cΔABF: Corrected change in aortic blood flow

cΔAC: Corrected change in aortic conductance

cΔHR: Corrected change in heart rate

cΔIBF: Corrected change in iliac blood flow

cΔIC: Corrected change in iliac conductance

cΔMAP: Corrected change in mean arterial pressure

cΔMBF: Corrected change in mesenteric blood flow

cΔMC: Corrected change in mesenteric conductance

cΔRBF: Corrected change in renal blood flow

cΔRC: Corrected change in renal conductance

D1: Dose 1

D2: Dose 2

D3: Dose 3

D4: Dose 4

G: Conductance

HR: Heart rate

IBF: Iliac blood flow

IC: Iliac conductance

LV+S: Left ventricle and septum

MAP: Mean arterial pressure

MBF: Mesenteric blood flow

MC: Mesenteric conductance

n: Sample size

NAD(P)H: Nicotinamide adenine dinucleotide phosphate

NAD^+: Nicotinamide adenine dinucleotide

PLA_2: Phospholipase A_2

PLC: Phospholipase C

PLD: Phospholipase D

Q: Flow

RAAS: Renin-angiotensin-aldosterone system

RBF: Renal blood flow

RC: Renal conductance

RV: Right ventricle

SO: Sham-operated

ΔABF: Change in aortic blood flow

ΔAC: Change in aortic conductance

ΔG: Change in conductance

ΔHR: Change in heart rate

ΔIBF: Change in iliac blood flow

ΔIC: Change in iliac conductance

ΔMAP: Change in mean arterial pressure

ΔMBF: Change in mesenteric blood flow

ΔMC: Change in mesenteric conductance

ΔQ: Change in flow

ΔRBF: Change in renal blood flow

ΔRC: Change in renal conductance

1. Introduction

1.1. The cardiovascular system and haemodynamics

The rat vasculature is composed of the pulmonary and systemic circulations. Each circuit originates at the heart with a large conducting artery that continually branches eventually forming smaller resistance arteries, then arterioles, and finally capillaries. Capillaries converge into venules, which merge to form veins. Eventually, the largest veins deliver blood back to the heart, which serves to propel blood throughout the vasculature. The right atrium and ventricle serve the pulmonary circulation, while those of the left heart serve the systemic circulation (Tortora and Derrickson, 2012).

1.1.1. Cardiac output

The volume of blood that is ejected from a ventricle with each cardiac cycle is referred to as stroke volume. Cardiac output refers to the volume of blood ejected from a ventricle in one minute. It is the product of stroke volume and heart rate (Tortora and Derrickson, 2012). Both stroke volume and heart rate are regulated by nervous and humoral mechanisms to maintain an appropriate cardiac output in changing circumstances (Silverthorn, 2009). Stroke volume is influenced by three factors: preload, contractility, and afterload (Tortora and Derrickson, 2012).

Preload refers to the ventricular stretch that is caused by the volume of blood in the ventricle immediately prior to contraction. To a limit, greater ventricular stretch elicits stronger ventricular contractions. As such, increasing the volume of blood in a ventricle causes greater ventricular stretch and more forcible contractions. This results in

a greater volume of blood being expelled from the ventricle (Tortora and Derrickson, 2012).

Preload is influenced by venous tone and heart rate. When venous tone increases, reserve blood is mobilized from the highly compliant venous vasculature and the pressure gradient driving venous flow is increased. This increases the venous return and ventricular preload, causing stroke volume and cardiac output to increase (Tortora and Derrickson, 2012). Decreased carotid sinus pressure, hypoxia, hypercapnia and circulating adrenaline and noradrenaline can increase venous return (Braunwald et al., 1963; Ross et al., 1961a). In contrast, increased carotid sinus pressure and elevated left ventricular pressure reduce venous return (Ross et al., 1961a; Ross et al., 1961b).

Contractility refers to the force with which the ventricles contract. Increasing contractility allows more blood to be ejected from the ventricle, increasing stroke volume and therefore cardiac output. Sympathetic nerves release noradrenaline onto the sinoatrial and atrioventricular nodes, as well as contractile fibers of the heart. At the nodes, noradrenaline causes an increase in the spontaneous depolarization of pacemaker cells, whereas it causes cardiomyocytes to increase contractility. Similarly, parasympathetic nerves release acetylcholine onto the nodes and contractile fibers of the heart. At the nodes, acetylcholine causes a decrease in the spontaneous depolarization of pacemaker cells. However, parasympathetic nerves exert little control over contractility. In addition, circulating adrenaline and noradrenaline, as well as thyroid hormones, increase heart rate and contractility (Tortora and Derrickson, 2012).

Afterload refers to the arterial pressure against which the heart ejects blood. If the afterload is elevated, the ventricle must generate greater pressure to open the semilunar valve and eject blood. Consequently, stroke volume is decreased. Afterload is affected by nervous and humoral factors that control vascular resistance, as well as structural changes that increase resistance to blood flow (Tortora and Derrickson, 2012).

Increasing heart rate can result in an increased cardiac output, as blood is ejected from the ventricles more frequently. However, heart rate determines the quantity of time available for ventricular filling. If the heart rate increases, ventricular filling and ventricular preload decrease resulting in a smaller stroke volume. A threshold heart rate exists above which ventricular filling is reduced to the point that cardiac output falls even though the heart is pumping rapidly (Tortora and Derrickson, 2012).

1.1.2. Mean arterial blood pressure

Mean arterial blood pressure is an estimate of the outward force applied on vessel walls by blood. It is the product of cardiac output and systemic vascular resistance, which is the force that opposes blood flow through the systemic circulation. The three major determinants of systemic vascular resistance are blood viscosity, blood vessel length, and blood vessel diameter. Vascular resistance is inversely proportional to the fourth power of blood vessel lumen diameter. As such, control of blood vessel diameter is very important; it is controlled by nervous and humoral factors (Tortora and Derrickson, 2012).

Neurogenic constriction of arterioles can occur in response to decreased pressure in the carotid or aortic blood vessels. The parasympathetic output to the heart is simultaneously decreased. Conversely, increased pressure acting on aortic or carotid baroreceptors reduces sympathetic arterial tone and increases parasympathetic output to the heart. Furthermore, the chemoreceptors of the carotid and aortic bodies increase sympathetic output in response to hypoxia, hypercapnia, and acidosis (Tortora and Derrickson, 2012; Silverthorn, 2009).

Blood pressure is also regulated by circulating factors. The sympathetic response elicited by carotid and aortic baroreceptors or chemoreceptors causes adrenaline and noradrenaline to be released from the adrenal medulla. The circulating catecholamines result in vasoconstriction (Tortora and Derrickson, 2012). Other circulating factors such as: atrial natriuretic peptide, angiotensin, vasopressin, endothelin, nitric oxide (NO), and bradykinin can result in vasoconstriction or dilation and therefore increase or decrease blood pressure (Tortora and Derrickson, 2012; Meens et al., 2009; Sampaio et al., 2007; Bisset and Lewis, 1962; review: Gassanov et al., 2011; Nguyen Dinh Cat and Touyz, 2011; Kurdi et al., 2005; Willenbrock et al., 1999a).

1.1.3. Vascular conductance

Vascular conductance is used as an indirect measure of vascular tone. Vascular resistance is the inverse of vascular conductance. Conductance is the better parameter to use if the observed change primarily affects blood flow, as the two are directly proportional and therefore linearly related. Conversely, if the vascular effects are

centered on changing blood pressure without significant change in flow, then vascular resistance is the better measurement. Choosing the appropriate parameter reduces the error associated with calculating average vascular conductance or resistance. Generally, conductance is the correct choice for *in vivo* experimentation (Lautt, 1989).

1.2. The renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone system (RAAS) serves to regulate blood pressure, blood volume, and renal function. Juxtaglomerular cells of renal afferent arterioles release renin into the circulation in response to: sympathetic stimulation, hypotension, reduced sodium and chloride load at the macula densa, hypovolemia, hypokalaemia, or reduced renal perfusion (review: De Mello and Frohlich, 2011; Nguyen Dinh Cat and Touyz, 2011; Salgado et al., 2010; Siragy and Carey, 2010; Kurdi et al., 2005). Renin, an aspartyl protease, catalyzes the conversion of angiotensinogen to angiotensin I (Ang I). Ang I is further processed into highly vasoactive angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE); chymase also catalyzes this reaction (Donoghue et al., 2000; review: Nguyen Dinh Cat and Touyz, 2011; Rabelo et al., 2011; Salgado et al., 2010; Kobori et al., 2007). Generally, Ang II binds to two receptors, the angiotensin II receptor type 1 (AT_1) and the angiotensin II receptor type 2 (AT_2; Figure 1; review: Zhuo et al., 1998).

In adults, AT_1 receptors predominate; they are responsible for the classic angiotensin effects. Activation of AT_1 receptors causes: vasoconstriction, aldosterone secretion, sodium reabsorption, thirst stimulation, sympathetic potentiation, cardiac

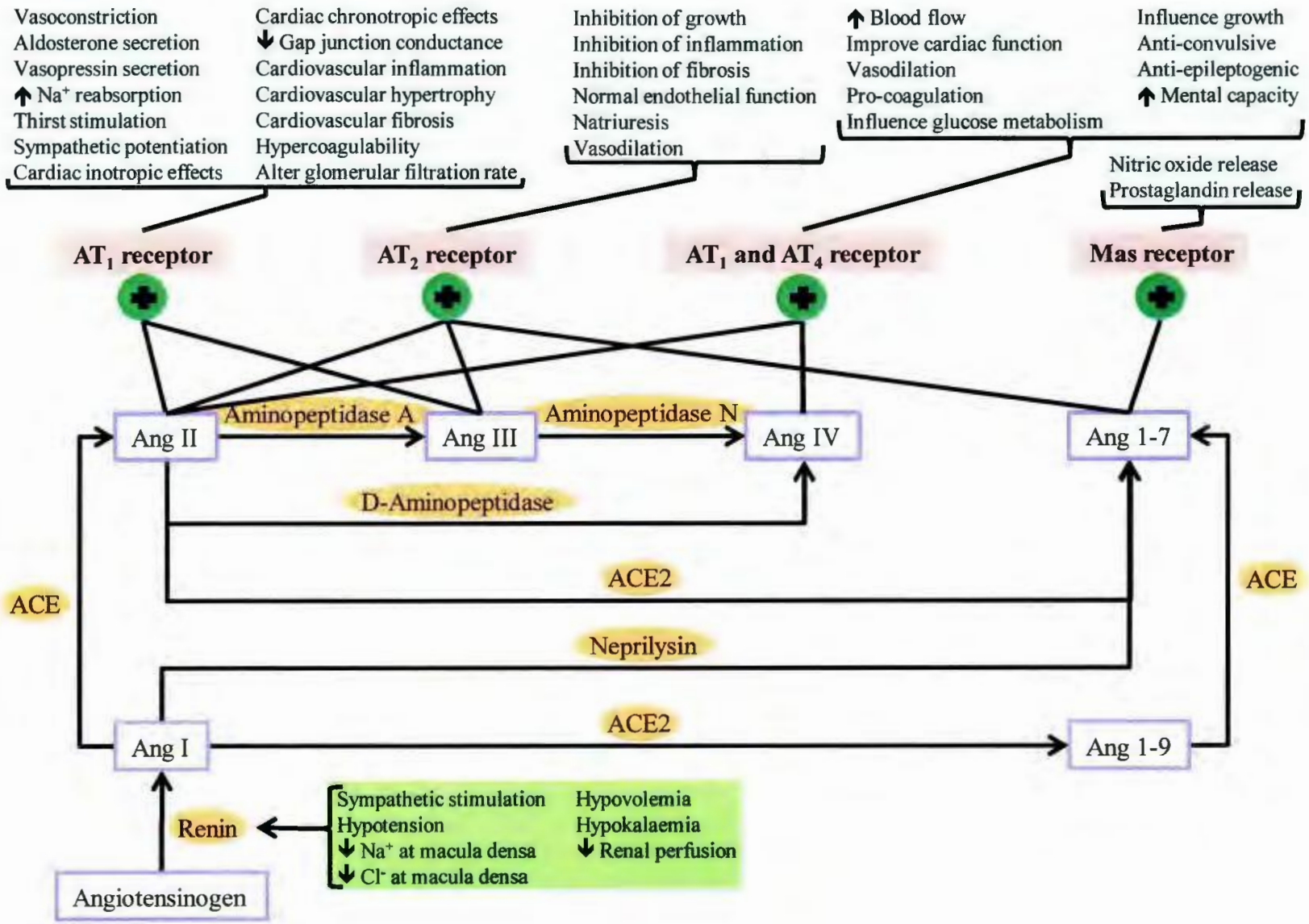
Vasoconstriction
Aldosterone secretion
Vasopressin secretion
↑ Na+ reabsorption
Thirst stimulation
Sympathetic potentiation
Cardiac inotropic effects
Cardiac chronotropic effects
↓ Gap junction conductance
Cardiovascular inflammation
Cardiovascular hypertrophy
Cardiovascular fibrosis
Hypercoagulability
Alter glomerular filtration rate
Inhibition of growth
Inhibition of inflammation
Inhibition of fibrosis
Normal endothelial function
Natriuresis
Vasodilation
↑ Blood flow
Improve cardiac function
Vasodilation
Pro-coagulation
Influence glucose metabolism
Influence growth
Anti-convulsive
Anti-epileptogenic
↑ Mental capacity
Nitric oxide release
Prostaglandin release
AT1 receptor
AT2 receptor
AT1 and AT4 receptor
Mas receptor
Ang II
Aminopeptidase A
Ang III
Aminopeptidase N
Ang IV
Ang 1-7
D-Aminopeptidase
ACE2
ACE
ACE
Neprilysin
ACE2
Ang I
Ang 1-9
Renin
Sympathetic stimulation
Hypotension
↓ Na+ at macula densa
↓ Cl- at macula densa
Hypovolemia
Hypokalaemia
↓ Renal perfusion
Angiotensinogen

Figure 1. Angiotensin activation cascade and receptor signaling.

Stimuli for activation of the angiotensin cascade are shown with a green background. Enzymes are shown in orange ovals. Peptides are shown in purple boxes. Receptors are shown in red rectangles. The consequences of receptor activation are shown with blue backgrounds. Green circles indicate activation of a receptor.

(Adapted from: De Mello and Frohlich, 2011; Nguyen Dinh Cat and Touyz, 2011; De Mello, 2010; Ocaranza et al., 2010; Salgado et al., 2010; Siragy and Carey, 2010; De Mello, 2009; Kobori et al., 2007; Sampaio et al., 2007; Duprez, 2006; Kurdi et al., 2005; De Mello, 2004; Donoghue et al., 2000; Zhuo et al., 1998)

inotropic effects, cardiac chronotropic effects, decreased cardiac gap junction conductance, cardiovascular inflammation, cardiovascular hypertrophy, cardiovascular fibrosis, hypercoagulability, alteration of glomerular filtration rate, and vasopressin secretion (review: Nguyen Dinh Cat and Touyz, 2011; Salgado et al., 2010; Siragy and Carey, 2010; De Mello, 2009; Kobori et al., 2007; Kurdi et al., 2005). Aldosterone increases sodium and water reabsorption in the collecting ducts, while vasopressin causes water reabsorption from the collecting ducts and vasoconstriction (review: Gassanov et al., 2011; Salgado et al., 2010; Chen and Schrier, 2006; Kurdi et al., 2005).

Expression of AT_2 receptors is greater during fetal development (notably: brain, kidneys, skin, aorta, and skeletal muscle) than during adulthood (Aguilera et al., 1994; review: Saavedra, 1992). However, expression of AT_2 receptors in adults tends to increase during: hypertension, myocardial infarction, heart failure, renal failure, cerebral ischemia, and diabetes, likely because AT_2 receptors typically mediate effects that oppose those of AT_1 receptors (review: Nguyen Dinh Cat and Touyz, 2011; Salgado et al., 2010; Duprez, 2006). Activation of AT_2 receptors can inhibit growth, inflammation, and fibrosis, as well as promote normal endothelial cellular function, natriuresis, and vasodilation (review: Nguyen Dinh Cat and Touyz, 2011; Salgado et al., 2010; Siragy and Carey, 2010; Duprez, 2006).

1.2.1. AT_1 receptor signaling in the cardiovascular system

Activation of AT_1 receptors can initiate multiple intracellular signaling cascades resulting in cardiac and vascular: contraction, inflammation, remodeling, and hypertrophy

(Figure 2; view: Higuchi et al., 2007; Mehta and Griendling, 2007; Touyz and Berry, 2002). The effects of AT_1 receptor activation are potentiated by dimerization with bradykinin B_2 receptors, and inhibited by dimerization with AT_2 receptors (review: Mehta and Griendling, 2007).

Activation of AT_1 receptors leads to dissociation of the G proteins G_α and $G_{\beta\gamma}$, and can initiate activation of phospholipases A_2, C, and D (Figure 2). Phospholipase A_2 releases arachidonic acid from membrane phospholipids. Arachidonic acid is converted into prostaglandin H_2 by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), and then further converted into various prostanoids. In a parallel pathway, lipoxygenases produce leukotrienes from arachidonic acid. Leukotriene intermediates can be oxidized by cytochrome P450, the products of which counter the effects of leukotrienes. Collectively, AT_1 receptor activation of phospholipase A_2 can cause: relaxation, contraction, inflammation, and growth (review: Mehta and Griendling, 2007; Touyz and Berry, 2002).

Phospholipase C activates two parallel pathways by cleaving membrane-derived phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol. Inositol 1,4,5-triphosphate mediates calcium release from the sarcoplasmic reticulum resulting in: calcium-calmodulin binding, myosin light chain kinase activation, and smooth muscle contraction. Diacylglycerol activates protein kinase C causing an increase in the intracellular pH and initiation of the Ras/Raf/MECK/ERK pathway. Ultimately, AT_1 receptor activation of phospholipase C can cause: contraction, growth, hypertrophy, and inhibition of apoptosis. Furthermore, diacylglycerol serves as a source of arachidonic

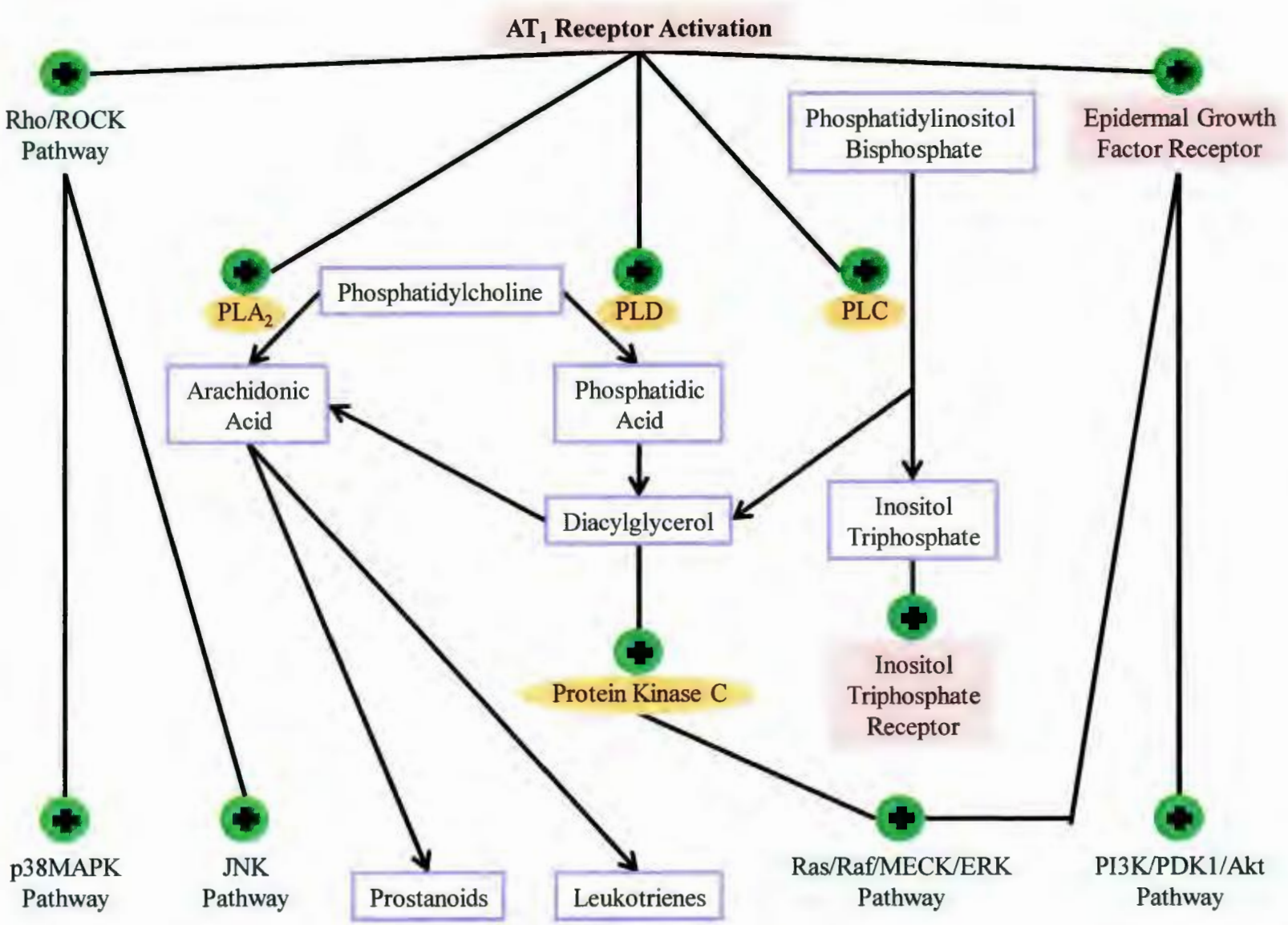
AT1 Receptor Activation
Rho/ROCK Pathway
Phosphatidylinositol Bisphosphate
Epidermal Growth Factor Receptor
PLA2
Phosphatidylcholine
PLD
PLC
Arachidonic Acid
Phosphatidic Acid
Diacylglycerol
Inositol Triphosphate
Protein Kinase C
Inositol Triphosphate Receptor
p38MAPK Pathway
JNK Pathway
Prostanoids
Leukotrienes
Ras/Raf/MECK/ERK Pathway
PI3K/PDK1/Akt Pathway

Figure 2. AT_1 receptor signal transduction.

Select enzymes are shown in orange ovals. Important signaling molecules are shown in purple boxes. Receptors are shown in red rectangles. Pathways are shown with a blue background. Green circles indicate activation of an enzyme, pathway, or receptor. PLA_2: phospholipase A_2; PLD: phospholipase D; PLC: phospholipase C.

(Adapted from: Higuchi et al., 2007; Mehta and Griendling, 2007; Touyz and Berry, 2002)

acid that joins the phospholipase A_2-activated signaling cascade ending in relaxation, contraction, inflammation, and growth (review: Mehta and Griendling, 2007; Touyz and Berry, 2002). Phospholipase D cleaves membrane-derived phosphatidylcholine into phosphatidic acid and choline, the former is converted to diacylglycerol and follows the phospholipase C signaling cascade to alter intracellular pH and initiate the Ras/Raf/MECK/ERK pathway (review: Mehta and Griendling, 2007).

Activation of AT_1 receptors and subsequent dissociation of G_α and $G_{\beta\gamma}$ can also activate the Rho/ROCK pathway. The G_α subunit can initiate activation of RhoGEFs, which in turn activate Rho by exchanging GTP for GDP. Rho then activates ROCK, which by reactive oxygen species (ROS)-dependent pathways activates JNK and p38MAPK. Together, the Rho/ROCK pathways result in: inflammation, hypertrophy, remodeling, migration, contraction, apoptosis, survival, and differentiation (review: Higuchi et al., 2007; Mehta and Griendling, 2007).

In addition, stimulation of AT_1 receptors can transactivate epidermal growth factor receptors (review: Higuchi et al., 2007). Activation of AT_1 receptors results in the production of ROS that allow for Src activation (review: Mehta and Griendling, 2007). In vascular smooth muscle cells, Src and Pyk2 are needed for ADAM17 to release an extracellular ligand for epidermal growth factor receptors (review: Higuchi et al., 2007; Mehta and Griendling, 2007). Binding its ligand induces a conformational change in the receptor, promotes dimerization, and initiates autophosphorylation (review: Mehta and Griendling, 2007). Epidermal growth factor receptors then activate two parallel pathways: PI3K/PDK1/Akt and Ras/Raf/ERK. Together, these pathways result in:

growth, remodeling, survival, differentiation, migration, adhesion, hypertrophy, and inflammation (review: Higuchi et al., 2007; Mehta and Griendling, 2007).

AT_1 receptor activation can activate nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidases that produce ROS. ROS act as signaling molecules and are necessary for signaling via p38MAPK, Akt, and epidermal growth factor receptors, as well as transcription factor activation. Combined, these pathways implicate ROS in: inflammation, survival, growth, and hypertrophy (review: Mehta and Griendling, 2007).

In endothelial cells, activation of AT_1 receptors results in NAD(P)H oxidase and endothelial nitric oxide synthase (eNOS) activation. Ideally, NO produced by eNOS would cause vasodilation. However, peroxynitrate can result from excessive production of ROS by NAD(P)H oxidase. ROS can cause eNOS uncoupling, in which eNOS inappropriately produces ROS. Excessive ROS can result in: protein damage, tetrahydrobiopterin deficiency, decreased intracellular nicotinamide adenine dinucleotide (NAD^+), and decreased intracellular adenosine triphosphate. In addition to the detrimental effects of reduced NO availability, activation of the p38MAPK pathway can induce adhesion molecule production, while the ERK pathway can cause endothelin production and consequent vasoconstriction and growth (review: Higuchi et al., 2007).

1.2.2. Angiotensin-converting enzyme II

The angiotensin-converting enzyme II (ACE2) is a homologue of ACE that catalyzes the conversion of Ang I to angiotensin 1-9 (Ang 1-9) and Ang II to angiotensin 1-7 (Ang 1-7; Ocaranza et al., 2010; Donoghue et al., 2000; review: Nguyen Dinh Cat

and Touyz, 2011; Rabelo et al., 2011; Salgado et al., 2010; Siragy and Carey, 2010; De Mello, 2009; Kurdi et al., 2005; De Mello, 2004). Through many mechanisms, ACE2 is believed to function in a protective role and to counteract the effects of ACE (Ocaranza et al., 2010; review: Rabelo et al., 2011; Siragy and Carey, 2010; De Mello, 2009; Kurdi et al., 2005). Importantly, ACE inhibitors do not inhibit ACE2 (Ocaranza et al., 2010; Donoghue et al., 2000; review: Siragy and Carey, 2010; De Mello, 2009).

ACE2 is mostly expressed in cardiac and renal endothelial cells, as well as the renal epithelium. However, it is also expressed in the lungs, gastrointestinal tract, and testes (Donoghue et al., 2000; review: De Mello, 2010; Salgado et al., 2010; Siragy and Carey, 2010; De Mello, 2009; Ferrario, 2006; Kurdi et al., 2005; De Mello 2004; Oudit et al., 2003). In contrast, ACE is more globally expressed in the vascular endothelium, as well as renal epithelial cells and cardiac cells (review: Nguyen Dinh Cat and Touyz, 2011; Siragy and Carey, 2010; De Mello, 2009; Kurdi et al., 2005). An isozyme of ACE is also expressed in the testes; it has only one catalytic site, whereas elsewhere ACE has two catalytic sites (Donoghue et al., 2000). Both ACE and ACE2 cleave amino acid residues from the carboxyl-terminal of their substrates (review: Nguyen Dinh Cat and Touyz, 2011; Rabelo et al., 2011; De Mello, 2010; Siragy and Carey, 2010; Salgado et al., 2010; De Mello, 2009; Kurdi et al., 2005). Both enzymes are membrane bound, although they can be cleaved from the membrane to circulate throughout the vasculature (Ocaranza et al., 2010; Donoghue et al., 2000; review: De Mello, 2009; Oudit et al., 2003).

Bradykinin is a peptide that can mediate vasodilation throughout the vasculature, at least partially via NO and arachidonic acid release following activation of bradykinin B_2 receptors (review: Manolis et al., 2010). It is degraded by ACE but not by ACE2 (Ocaranza et al., 2010; Donoghue et al., 2000; review: Kurdi et al., 2005). However, ACE2 can hydrolyze peptides other than Ang I and Ang II (Vickers et al., 2002; Donoghue et al., 2000). It was observed to completely hydrolyze samples of: apelin-36, apelin-13, dynorphin A 1-13, neocasomorphin, β-casomorphin, and des-Arg^9-bradykinin *in vitro*. Furthermore, *in vitro* samples of Lys-des-Arg^9-bradykinin, ghrelin, and neurotensin 1-8 were partially hydrolyzed by ACE2 (Vickers et al., 2002). Donoghue et al. (2000) also reported that ACE2 could hydrolyze kinetensin.

Des-Arg^9-bradykinin mediates vasodilation by activation of bradykinin B_1 receptors that are expressed during tissue damage and inflammation; the peptide is inactivated by ACE2 (Donoghue et al., 2000). Apelin-13 and apelin-36 increase contractility, as well as influence vascular tone and fluid balance, by activating apelin receptors (Vickers et al., 2002; review: Chandrasekaran et al., 2008). The receptors are expressed throughout the body, including vascular endothelial cells, vascular smooth muscle, and cardiomyocytes. *In vivo* studies have reported variable species-dependent effects of apelin infusion on blood pressure. Similarly, variable effects of apelin receptor activation on the plasma concentration of vasopressin have been reported. However, *in vitro* studies suggest that apelin receptors mediate vasodilation via NO production (review: Chandrasekaran et al., 2008).

1.2.3. Angiotensin III and IV

Aminopeptidase A can catalyze the conversion of Ang II into angiotensin III (Ang III); the latter peptide can bind both AT_1 and AT_2 receptors. *In vivo*, it causes vasoconstriction, aldosterone release, and raises blood pressure. *In vitro*, Ang III has been observed to mediate cellular growth, inflammation, and extracellular matrix protein production (review: Nguyen Dinh Cat and Touyz, 2011).

Aminopeptidase N can catalyze the conversion of Ang III into angiotensin IV (Ang IV); similarly, D-aminopeptidase can convert Ang II to Ang IV. Ang IV mediates a range of effects via AT_1 and angiotensin IV receptors (AT_4). It has been observed to increase blood flow, improve cardiac function, and cause vasodilation (review: Nguyen Dinh Cat and Touyz, 2011). Ang IV may also act as a pro-coagulant and influence glucose metabolism and cellular growth (review: Salgado et al., 2010).

AT_4 receptors are notably expressed in the endothelium (review: Duprez, 2006). Activation of AT_4 receptors by Ang IV or Ang II, or AT_1 receptors by Ang II, increases the expression of plasminogen activator inhibitor-1, a factor involved in thrombosis and inflammation (Mehta et al., 2002; review: Suzuki et al., 2011; Salgado et al., 2010; Duprez, 2006).

1.2.4. Angiotensin 1-9

In addition to ACE2, cathepsin A can also produce Ang 1-9 (Ocaranza et al., 2010; review: Nguyen Dinh Cat and Touyz, 2011; Rabelo et al., 2011; Salgado et al., 2010; Siragy and Carey, 2010; Kurdi et al., 2005). Although this peptide may not have a

direct effect on vascular tone, it can exert an indirect influence on haemodynamics (review: Salgado et al., 2010). Simplistically, conversion of Ang I to Ang 1-9 by ACE2 might reduce the available quantity of Ang I for conversion to Ang II by ACE. In ACE2 knockout mice, Crackower et al. (2002) found increased concentrations of renal and cardiac Ang I, as well as increased concentrations of renal, cardiac, and plasma Ang II. The increased Ang II concentrations were not produced by increased ACE transcription. Unfortunately, it was not determined if Ang I expression was increased or if the lack of ACE2 competition for the Ang I substrate resulted in the increased concentrations. It is likely that both reduced competition for Ang I and reduced ACE2 degradation of Ang II are responsible for the increased concentrations of Ang II.

ACE2 competes with ACE for Ang I as a substrate, and the ACE2 product of Ang I hydrolysis is a competitive inhibitor of ACE (Ocaranza et al., 2010; Donoghue et al., 2000). Ang 1-9 is further hydrolyzed by ACE to form Ang 1-7 (Ocaranza et al., 2010; Donoghue et al., 2000; review: Nguyen Dinh Cat and Touyz, 2011). This competitive inhibition would decrease the rate of other reactions catalyzed by ACE, namely Ang II formation and bradykinin degradation (Ocaranza et al., 2010; review: Kurdi et al., 2005). Chronic administration of Ang 1-9 following induction of myocardial infarction in male Sprague-Dawley rats reduced the plasma concentration of Ang II as well as plasma and left ventricular ACE activity (Ocaranza et al., 2010). Furthermore, beyond competitively inhibiting the preferential degradation of bradykinin by ACE, Ang 1-9 was observed to potentiate ACE-resistant bradykinin B_2 receptor agonist mediated arachidonic acid and NO release in cultured human pulmonary artery endothelial cells. This potentiation was

not produced by Ang 1-9 competitive inhibition of ACE and a consequent maintenance of agonist concentration, as the concentration of Ang 1-9 was too low to inhibit ACE and the B_2 receptor agonist was resistant to ACE degradation (Jackman et al., 2002).

Ang 1-9 demonstrates anti-hypertrophic effects following myocardial infarction. Chronic administration of Ang 1-9 to male Sprague-Dawley rats following coronary artery ligation prevented left ventricular hypertrophy, as observed by decreased indices of left ventricular size measured *in situ* by echocardiography and by weight following excision of the heart. Conversion of Ang 1-9 into Ang 1-7 by ACE and subsequent binding to the Ang 1-7 receptor, Mas, did not mediate the anti-hypertrophic effect, as the addition of a Mas receptor antagonist did not reduce the effectiveness of Ang 1-9 administration. However, the authors concede that Ang 1-7 can act on AT_2 receptors, and that they did not simultaneously antagonize this receptor. Therefore, the anti-hypertrophic effect could be produced by Ang 1-9 conversion to Ang 1-7 by ACE, followed by Ang 1-7 binding to AT_2 receptors. In other experiments, enalapril (ACE inhibitor) and candesartan (AT_1 receptor antagonist) increased the plasma Ang 1-9 concentration when administered chronically following myocardial infarction. This suggests that the anti-hypertrophic effects of these drugs might be partially attributable to their ability to increase the concentration of circulating Ang 1-9 (Ocaranza et al., 2010).

1.2.5. Angiotensin 1-7

In addition to ACE2 production of Ang 1-7 from Ang II, neprilysin can convert Ang I to Ang 1-7 (Ocaranza et al., 2010; review: Nguyen Dinh Cat and Touyz, 2011; De

Mello, 2011; Rabelo et al., 2011; De Mello, 2010; Salgado et al., 2010; Siragy and Carey, 2010; Kurdi et al., 2005; De Mello, 2004). Ang 1-7 binds Mas receptors that are distributed throughout the circulation, brain, heart, kidneys, liver, and testes (review: Nguyen Dinh Cat and Touyz, 2011; Rabelo et al., 2011; De Mello, 2010; De Mello, 2009; Kurdi et al., 2005; De Mello, 2004). However, Ang 1-7 can also bind AT_2 receptors (Ocaranza et al., 2010; review: Rabelo et al., 2011).

Together, the ACE2/Ang 1-7/Mas axis seems to oppose the ACE/Ang II/AT_1 axis by reducing the concentration of available Ang II and through Mas receptor activation causing NO and prostaglandin release (Sampaio et al., 2007; review: Rabelo et al., 2011; De Mello, 2009; Kurdi et al., 2005). NO is produced in response to Mas receptor activation by PI3K/PKB/Akt dependent phosphorylation of serine 1177 and dephosphorylation of threonine 495 of eNOS. NO inhibits platelet aggregation, platelet adhesion, vascular smooth muscle proliferation, and mediates vasodilation (Sampaio et al., 2007). Ang 1-7 has also been observed to prevent cardiac remodeling and hypertrophy (Donoghue et al., 2000; review: De Mello, 2009; Kurdi et al., 2005). It is possible for Ang 1-7 activated Mas receptors to dimerize with AT_1 receptors, thereby inhibiting activation of the AT_1 receptors (review: Nguyen Dinh Cat and Touyz, 2011).

Ang 1-7 is believed to counter the effects of Ang II during myocardial ischaemia and reduce the risk of arrhythmias during reperfusion (review: De Mello, 2010; De Mello, 2009; Kurdi et al., 2005; De Mello, 2004). Activation of cardiac AT_1 receptors by Ang II decreases electrical conductance through ventricular gap junctions in hamsters. Similarly, intracellular administration of Ang II decreases cardiac gap junction

conductance (De Mello, 1996). Furthermore, Ang II and aldosterone induce inflammation and interstitial fibrosis that further decrease electrical conduction throughout the heart. Treatment with enalapril (ACE inhibitor) was observed to increase intercellular coupling. Ang 1-7 demonstrated antiarrhythmic properties when administered to isolated ventricular myocytes of cardiomyopathic hamsters; it caused hyperpolarization, increased conduction velocity, and increased refractoriness. However, high doses of exogenous Ang 1-7 were proarrhythmic (De Mello et al., 2007).

In heart failure, extracellular Ang II increases cellular volume by inhibiting the sodium pump and activating the sodium-potassium-2-chloride transporter. Ang II induced swelling also activates the aforementioned chloride current. In contrast, application of Ang 1-7 to cells swollen by previous exposure to a hypotonic solution activates the sodium pump and inhibits the chloride current, thereby reducing cellular volume (review: De Mello, 2010). Similarly, intracellular injection of Ang II produces the same results (De Mello, 2008; review: De Mello, 2010). Therefore, the antiarrhythmic actions of Ang 1-7 may be related to a reduction in cellular swelling (review: De Mello, 2010).

The beneficial effects of Ang 1-7 may also result from a reduction in ROS. Superoxide, a ROS, acts as a scavenger of excess NO; the reaction between superoxide and NO produces peroxynitrate. Excessive production of peroxynitrate is detrimental, it is known to participate in atherogenesis by oxidizing low-density lipoprotein. In addition, excess superoxide can decrease the availability of NO necessary to mediate vascular smooth muscle relaxation. As such, superoxide production for NO scavenging must be controlled (review: Rabelo et al., 2011).

Activation of the Mas receptor by Ang 1-7 increases eNOS activity; increased production of NO counteracts the effect of excessive superoxide production. Mas deficient mice were observed to have decreased superoxide dismutase and catalase activity, this was accompanied by increased levels of ROS. Similarly, lipid peroxidation and other measures of oxidative stress were increased in mice that did not express Mas receptors. In these mice, NAD(P)H oxidase activity was also increased. In contrast, increased expression of ACE2 in the vascular smooth muscle of spontaneously hypertensive stroke prone rats improved endothelial function and reduced the extent of Ang II induced vasoconstriction. Similarly, chronic administration of Ang 1-7 to diabetic spontaneously hypertensive rats improved renal artery endothelial function and decreased renal NAD(P)H oxidase transcription and activity (Benter et al., 2008). Ang 1-7 also reduced Ang II induced NAD(P)H oxidase activity in human endothelial cells (review: Rabelo et al., 2011). However, it has also been suggested that Ang 1-7 stimulates oxidative stress in the kidney (Gonzales et al., 2002). With respect to disease, chronic administration of Ang 1-7 seems to protect against atherosclerosis in a mouse model of the disease, and protect endothelial cells from Ang II induced macrophage infiltration in atherosclerosis (review: Rabelo et al., 2011).

1.2.6. Local renin-angiotensin-aldosterone systems

Proteins and peptides of the RAAS are found extravascularly in tissues throughout the body, they may be extracellular or intracellular, and may be obtained from the circulation or synthesized locally. Of particular interest are the local RAAS of the

vasculature and heart. Angiotensin, ACE, Ang I, Ang II, as well as AT_1 and AT_2 receptors are found in cardiac and vascular tissue. Furthermore, the counter-regulatory Ang 1-7/Mas axis is also present in these tissues. Most of the local RAAS components are synthesized in the tissue, although the local synthesis of renin is debated. Also contributing to the local RAAS are the components that are produced in perivascular adipose tissue, as well as the components synthesized by immune cells in the vascular wall. Aside from acting on vascular tissues, the local RAAS may act directly on vascular neurons to influence discharge and reuptake of noradrenaline (review: Nguyen Dinh Cat and Touyz, 2011).

The local RAAS can operate independently of the systemic RAAS. Local concentrations of angiotensin peptides can be greater than that in the plasma, this is true of Ang II in hypertension. In fact, the local system may be active while the systemic system is suppressed (review: Nguyen Dinh Cat and Touyz, 2011). Increased sodium intake suppresses the systemic RAAS, but activates the cardiac RAAS (review: De Mello and Frohlich, 2011). Similarly, Ang II activation of AT_1 receptors of juxtaglomerular cells reduces renin release from these cells into the circulation, whereas activation of AT_1 receptors in the collecting ducts increases local renin release (review: Siragy and Carey, 2010).

Active local RAAS can produce local effects without activation of the systemic RAAS. Increased sodium intake can cause structural changes in the heart before blood pressure increases. AT_1 receptor antagonists can prevent this sodium induced cardiac remodeling (review: De Mello and Frohlich, 2011). Similarly, hypertensive patients

treated with β-blockers experienced similar decreases in blood pressure as those treated with ACE inhibitors or AT_1 antagonists. However, only the ACE inhibitors and AT_1 antagonists reversed vascular remodeling. It is possible that the continuation of vascular remodeling in those treated with β-blockers is produced by ongoing local RAAS activity independent of reduced blood pressure. Also, hypertensive patients with normal plasma renin activity experience a decrease in blood pressure when treated with ACE inhibitors or AT_1 receptor antagonists suggesting an active local and inactive systemic RAAS (review: Nguyen Dinh Cat and Touyz, 2011). Finally, in a transgenic mouse model with decreased plasma renin concentration, AT_1 receptor-dependent cardiac hypertrophy was observed without an increase in blood pressure (Mazzolai et al., 1998).

The (pro)renin receptor binds both prorenin and renin, it is expressed throughout the body. Activation of this receptor allows prorenin to function as renin without undergoing hydrolysis. As such, tissues that do not express renin can obtain prorenin from the circulation and then produce Ang II (review: Siragy and Carey, 2010). In mesangial cells, the receptor was observed to exert a profibrotic influence independent of Ang II, as shown in the presence of renin and ACE inhibitors, or an AT_1 antagonist (Huang et al., 2006). However, receptor expression is decreased upon excessive activation of (pro)renin receptors (review: Siragy and Carey, 2010). Aside from activated (pro)renin receptor hydrolysis of angiotensinogen, another way in which tissues can avoid renin-dependent activation of the angiotensin cascade is with proangiotensin-12. This peptide, observed in rat aorta, can induce vasoconstriction following hydrolysis by

chymase and ACE to form Ang I and then Ang II (Bujak-Gizycka et al., 2010; review: Nguyen Dinh Cat and Touyz, 2011).

1.3. Rats with aortocaval fistulae: an experimental model of heart failure

Heart failure describes a situation in which the heart cannot deliver sufficient blood to tissues throughout the vasculature. Rats with aortocaval fistulae undergo functional and structural cardiovascular changes similar to those of low-output congestive heart failure in humans (review: Abassi et al., 2011). In fact, Melenovsky et al. (2011) noted that sixty-five percent of rats with aortocaval fistulae displayed visible signs of heart failure, including lethargy and labored breathing, at twenty-two weeks after surgery. Some studies conducted within two weeks of introduction of aortocaval fistulae have divided rats with aortocaval fistulae into groups of sodium-excreting compensated and sodium-retaining decompensated rats, the latter tend to survive for less than two weeks (Abassi et al., 2005; Abassi et al., 1998a; Abassi et al., 1998b; Pieruzzi et al., 1995; Abassi et al., 1994; Hoffman et al., 1991; Abassi et al., 1990; Winaver et al., 1988). Sodium-retaining decompensated rats develop dyspnea, ascites, edema, and plural effusions (Abassi et al., 1994). Other rats with aortocaval fistulae survive longer (Melenovsky et al., 2011; Guo and Tabrizchi, 2008).

1.3.1. Haemodynamic effects of aortocaval fistulae

Aortocaval fistulae allow aortic blood to bypass the systemic circulation and flow directly into the inferior vena cava. The low resistance pathway from the arterial to the

venous vasculature significantly reduces total peripheral resistance (Ruzicka et al., 1993; Huang et al., 1992; Liu et al., 1991; Flaim et al., 1980). Increased mean circulatory filling pressure and decreased resistance to venous return have been observed in rats with aortocaval fistulae (Guo and Tabrizchi, 2008). Consequently, right atrial pressure is elevated in these rats (Willenbrock et al., 1999a; Willenbrock et al., 1999b; Huang et al., 1992).

Increased venous return results in an increased cardiac output (Melenovsky et al., 2011; Guo and Tabrizchi, 2008; Ruzicka et al., 1993; Huang et al., 1992; Liu et al., 1991; Flaim et al., 1980; Flaim et al., 1979). The majority of studies suggest that the presence of an aortocaval fistula has no effect on heart rate; therefore, increased cardiac output results from increased stroke volume (Hutchinson et al., 2011; Melenovsky et al., 2011; Guo and Tabrizchi, 2008; Willenbrock et al., 1999b; Ruzicka et al., 1993; Huang et al., 1992; Hoffman et al., 1991; Liu et al., 1991; Flaim et al., 1980). In time, blood volume increases to support the hyperdynamic circulation; there is a measurable decrease in hematocrit (Guo and Tabrizchi, 2008; Huang et al., 1992). A normal mean arterial pressure is maintained (Chen et al., 2011; Hutchinson et al., 2011; Melenovsky et al., 2011; Guo and Tabrizchi, 2008; Willenbrock et al., 1999a; Willenbrock et al., 1999b; Abassi et al., 1994; Huang et al., 1992; Flaim et al., 1979).

Left ventricular contractility is reduced in rats with aortocaval fistulae (Chen et al., 2011; Hutchinson et al., 2011; Melenovsky et al., 2011; Liu et al., 1991). However, Liu et al. (1991) noted that the rate of change in right ventricular pressure is elevated in these rats. In addition, several measurements suggest diastolic dysfunction in rats with

aortocaval fistulae (Chen et al., 2011; Gladden et al., 2011; Hutchinson et al., 2011; Melenovsky et al., 2011; Ruzicka et al., 1993; Liu et al., 1991; Flaim et al., 1980; Flaim et al., 1979).

Generally, intestinal blood flow is normal in rats with aortocaval fistulae (Huang et al., 1992). However, Flaim et al. (1979) measured reduced jejunal, but normal ileac and colonic blood flows at eight weeks after introduction of aortocaval fistulae. Similarly, decreased gastric blood flow has been observed, while splenic, hepatic and skeletal muscle blood flows remain normal (Huang et al., 1992; Flaim et al., 1980; Flaim et al., 1979).

Renal blood flow is reduced in rats with aortocaval fistulae (Bishara et al., 2008; Brodsky et al., 1998; Abassi et al., 1997; Huang et al., 1992). Specifically, it was observed that cortical perfusion is reduced, while medullary blood flow is maintained at a normal rate (Abassi et al., 2001a; Abassi et al., 1998a). The altered blood flow might result from changes in the expression of vasoactive substances or receptors. For instance, transcription of ET-1 and endothelin converting enzyme mRNA is increased in the renal cortex of sodium-retaining decompensated rats, while the medullary transcription of both proteins is similar in all rats (Abassi et al., 2001b; Abassi et al., 1998b). Also, cortical and medullary eNOS mRNA transcription is increased in sodium-retaining decompensated rats, with elevated eNOS protein expression observed in the renal medulla (Abassi et al., 2001b; Abassi et al., 1998a). Finally, although medullary COX-1 expression is similar in rats with and without aortocaval fistulae, COX-2 expression is upregulated in sodium-retaining decompensated rats (Abassi et al., 2001a).

1.3.2. Cardiac remodeling in rats with aortocaval fistulae

Chen et al. (2011) have suggested that an early cardiac inflammatory response to aortocaval fistulae induced haemodynamic alterations is responsible for cardiac remodeling. At three days after introduction of aortocaval fistulae, there is a concomitant increase in cardiac mast cell density, as well as the left ventricular protein expression of TNF-α and its receptors (McLarty et al., 2012; Melendez et al., 2011). There is also a simultaneous increase in left ventricular COX-1 and -2 protein expression, as well as a decrease in β1-integrin protein expression. Downstream products of COX-1 and -2 have been implicated in cardiac fibrosis, while β1-integrins anchor cells to the extracellular matrix thereby influencing cardiac structure and contractile coupling (McLarty et al., 2012). At two weeks after the introduction of aortocaval fistulae, cardiac mast cell density normalizes, yet matrix metalloprotease-2 and inflammatory pathways remain activated with elevated markers of oxidative stress and apoptosis (Lu et al., 2012; Chen et al., 2011). Cardiac collagen content is reduced by the fourth week after introduction of aortocaval fistulae and remains reduced (Chen et al., 2011; Hutchinson et al., 2011). By the fifth week after introduction of aortocaval fistulae, cardiac apoptosis, inflammation, and oxidative stress seem to normalize (Chen et al., 2011). However, further indications of ventricular remodeling have been noted at eight and fifteen weeks after introduction of aortocaval fistulae (Chen et al., 2011; Hutchinson et al., 2011).

Several genes have been identified as being differentially expressed in the left ventricle of rats with aortocaval fistulae compared to sham operated rats. Of particular interest, cardiac expression of inflammatory response genes were upregulated, while the

expression of genes encoding components of fatty acid and carbohydrate metabolism were downregulated (McLarty et al., 2012; Chen et al., 2011; Melenovsky et al., 2011). These findings are consistent with the general state of inflammation and the observation of impaired mitochondrial respiration in rats with aortocaval fistulae (Gladden et al., 2011).

Rats with aortocaval fistulae develop left and right ventricular hypertrophy (Lu et al., 2012; McLarty et al., 2012; Chen et al., 2011; Hutchinson et al., 2011; Melendez et al., 2011; Melenovsky et al., 2011; Guo and Tabrizchi, 2008; Willenbrock et al., 1999b; Ruzicka et al., 1993; Liu et al., 1991; Flaim, 1982; Flaim et al., 1979). Liu et al (1991) determined that ventricular enlargement is produced by parallel increases in myocyte length and width, with no change in the ratio of length to width. Alexander and Imbembo (1989) stated that the cardiovascular effects of aortocaval fistulae depend on: the size of fistula, the rate of fistula flow, the caliber of blood vessels involved, the proximity to the heart, and the time period that the fistula has been present. To test the effects of varying aortocaval fistula size, Ruzicka et al. (1993) introduced aortocaval fistulae with eighteen or twenty-gauge needles. They observed that right ventricular hypertrophy was significantly greater in rats with larger aortocaval fistulae. Atrial enlargement has also been noted in these rats (Willenbrock et al., 1999b).

1.3.3. The renin-angiotensin-aldosterone system in rats with aortocaval fistulae

The RAAS is activated in rats with aortocaval fistulae as evidenced by increased plasma renin activity (Abassi et al., 2001b; Pieruzzi et al., 1995; Abassi et al., 1994;

Ruzicka et al., 1993; Huang et al., 1992; Abassi et al., 1990; Winaver et al., 1988). Further supporting the idea that fistula size influences its cardiovascular effects, it has been demonstrated that larger fistulas result in greater plasma renin activity (Ruzicka et al., 1993). Similarly, plasma renin activity is increased in sodium-retaining decompensated, but not sodium-excreting compensated, rats with aortocaval fistulae (Abassi et al., 2001b; Abassi et al., 1994). Although plasma renin activity was increased, Pieruzzi et al. (1995) did not measure increased plasma ACE activity. Regardless, rats with aortocaval fistulae have an increased plasma concentration of Ang II (Willenbrock et al., 1999b). In addition, plasma aldosterone concentration was elevated in sodium-retaining decompensated, but not sodium-excreting compensated, rats with aortocaval fistulae (Pieruzzi et al., 1995; Winaver et al., 1988). Furthermore, plasma concentrations of: epinephrine, norepinephrine and vasopressin are elevated in rats with aortocaval fistulae (Bishara et al., 2008; Abassi et al., 2001b; Flaim et al., 1979).

1.3.4. The local renin-angiotensin-aldosterone system in rats with aortocaval fistulae

The local RAAS is activated in rats with aortocaval fistulae. Increased cardiac transcription of renin mRNA and increased left ventricular renin activity have been measured (Pieruzzi et al., 1995; Ruzicka et al., 1993). Similarly, cardiac transcription and expression of ACE mRNA is upregulated in rats with aortocaval fistulae, while that of ACE2 is reduced (Karram et al., 2005; Pieruzzi et al., 1995). Furthermore, transcription of AT_1 receptor mRNA is increased in sodium-retaining decompensated rats. However, transcription of AT_2 receptor mRNA is unaltered by the presence of an aortocaval fistula.

Similarly, the local renal RAAS may also be activated in rats with aortocaval fistulae. Increased renal transcription of renin mRNA has been observed, although transcription and protein expression of ACE is unchanged in rats with aortocaval fistulae. Similarly, no change in the transcription of AT_1 or AT_2 receptors was noted (Pieruzzi et al., 1995).

1.3.5. Natriuretic peptides in rats with aortocaval fistulae

Compensatory mechanisms may act to reduce the effects of RAAS activation (Winaver et al., 1988; review: Abassi et al., 2011). The plasma concentration of natriuretic peptides is elevated in rats with aortocaval fistulae (Abassi et al., 2001b; Willenbrock et al., 1999a; Willenbrock et al., 1999b; Pieruzzi et al., 1995; Abassi et al., 1994; Huang et al., 1992; Hoffman et al., 1991). Interestingly, the plasma concentration of B-type natriuretic peptide is significantly higher in sodium-retaining decompensated rats than in sodium-excreting compensated rats (Hoffman et al., 1991). Furthermore, left and right ventricular and atrial transcription of atrial natriuretic peptide mRNA is increased in rats with aortocaval fistulae, as is the absolute peptide content in all but the right ventricle. However, there is no increase in concentration when normalized to chamber weight (Willenbrock et al., 1999b). Similarly, cardiac B-type natriuretic peptide mRNA transcription is increased in rats with aortocaval fistula (Hutchinson et al., 2011).

1.3.6. Experimental findings on renal blood flow in rats with aortocaval fistulae

Renal blood flow is regulated by many factors in rats with aortocaval fistulae. Acute treatment with eprosartan, an AT_1 receptor antagonist, increased renal blood flow

to a greater extent and duration in rats with aortocaval fistulae than in sham-operated rats. A similar effect was observed with respect to cortical but not medullary blood flow, suggesting that Ang II acting on AT_1 receptors in the renal cortex might favor medullary perfusion in rats with aortocaval fistulae (Brodsky et al., 1998).

Furthermore, expression of ET-1, ET-1 receptors, and eNOS in the renal medulla is greater than in the cortex. This might be responsible for the normal baseline medullary blood flow and augmented medullary vasodilatory response to acutely administered exogenous ET-1. Baseline cortical perfusion is reduced in rats with aortocaval fistulae and declines with ET-1 infusion (Abassi et al., 1998a). Given the importance of the cortex in renal function, Brodsky et al. (1998) suggested that improved cortical perfusion is responsible for the increased glomerular filtration observed with eprosartan treatment. In light of this, Abassi et al. (1998a) noted that increased expression of ET-1 and eNOS in the renal cortex of sodium-retaining decompensated rats might explain both the reduced baseline cortical perfusion and reduced constrictive response to ET-1 infusion.

Finally, in rats with aortocaval fistulae, acute administration of an Ang II receptor antagonist improved the otherwise impaired renal vasodilation in response to endothelium-dependent or –independent NO production. Given that the response to, and not production of, NO was impaired, the authors suggested that impairment of NO mediated renal vasodilation might also contribute to decreased renal perfusion in rats with aortocaval fistulae (Abassi et al., 1997).

1.3.7. Experimental findings on cardiac remodeling in rats with aortocaval fistulae

Chronic administration of the ACE inhibitor enalapril had no effect on the development of cardiac hypertrophy in rats with aortocaval fistulae (Ruzicka et al., 1993). In contrast, chronic administration of the AT_1 receptor antagonist losartan significantly decreased the extent of left and right ventricular hypertrophy (Brodsky et al., 1998; Ruzicka et al., 1993). It has been suggested that cardiac hypertrophy in the presence of ACE inhibition might result from ACE-independent Ang II production (Ruzicka et al., 1993). However, it is also possible that ACE inhibition was inadequate at the tissue level.

Chronic administration of spironolactone, an aldosterone receptor antagonist, increased cardiac ACE2 expression and attenuated short-term cardiac hypertrophy. Chronic treatment with eprosartan, however, provided longer lasting anti-hypertrophic effects. Although spironolactone increased ACE2 expression, only eprosartan increased the activity of cardiac ACE2 (Karram et al., 2005). Similarly, chronic administration of the vasopressin V_2 receptor antagonist SR 121463B reduced cardiac hypertrophy, perhaps produced by haemodynamic improvements related to decreasing blood volume (Bishara et al., 2008).

In vitro, cardiac mast cell activation by substance P was inhibited by neurokinin-1 receptor antagonism. In rats with aortocaval fistulae, chronic neurokinin-1 receptor antagonism prevented left ventricular collagen degradation, mast cell recruitment, and TNF-α expression; it is suggested that the sensory nerves that release substance P are in

part responsible for the inflammatory response that initiates cardiac remodeling in rats with aortocaval fistulae (Melendez et al., 2011).

McLarty et al. (2012) noted that chronic estrogen treatment in male rats, started before the introduction of aortocaval fistulae, prevented or reduced the left ventricular expression of inflammatory genes. The expression of TNF-α, TNF-α receptor I, as well as COX-1 and -2 enzymes were reduced, while expression of TNF-α receptor II and β1-integrin were increased. It was suggested that the cardioprotective actions of estrogen might be related to this favorable change in gene expression. Furthermore, Lu et al. (2012) showed that chronic mast cell stabilization, prior to introduction of aortocaval fistulae, maintained normal left ventricular mast cell density, collagen content, and TNF-α concentration in female rats without ovaries. This suggests a link between estrogen and mast cell stabilization in the protection from myocardial remodeling produced by aortocaval fistulae in female rats.

1.3.8. Experimental findings on natriuretic peptides in rats with aortocaval fistulae

In sodium-retaining decompensated rats with aortocaval fistulae, the natriuretic response to acute infusion of atrial natriuretic peptide is impaired. Chronic administration of enalapril for one-week significantly increased daily sodium excretion, as well as improved the natriuretic response to acute treatment with atrial natriuretic peptide (Abassi et al., 1990; Winaver et al., 1988). Chronic treatment with losartan had similar effects (Abassi et al., 1994). Brodsky et al. (1998) noted that AT_1 receptor antagonists might improve sodium excretion by: increasing renal blood flow, increasing glomerular

filtration rate, reducing tubular sodium reabsorption, and reducing aldosterone secretion. Similarly, it was noted that acute treatment with an ACE inhibitor or Ang II receptor antagonist restored standard responses to an acute increase in intravascular volume (Willenbrock et al., 1999b). Collectively, these results suggest that the RAAS can overwhelm the effects of atrial natriuretic peptide. However, the impaired atrial natriuretic system is important in maintaining glomerular filtration rate in rats with aortocaval fistulae, as evidenced by the exaggerated effect of natriuretic peptide antagonism on glomerular filtration rate (Willenbrock et al., 1999a).

1.3.9. Experimental findings on bio-energetics in rats with aortocaval fistulae

Aside from structural impairment of cardiac function, Gladden et al. (2011) suggest bio-energetic impairment of the heart in rats with aortocaval fistulae. They noted that left ventricular xanthine oxidase activity is elevated in rats with aortocaval fistulae, while mitochondrial respiration and cardiac function are impaired. When modeled in isolated left ventricular cardiomyocytes, pretreatment with a mitochondrial antioxidant prevented the increase in xanthine oxidase activity, suggesting that mitochondria derived ROS activate xanthine oxidase. Allopurinol, a xanthine oxidase inhibitor, improved mitochondrial respiration and cardiac function of rats with aortocaval fistulae. As xanthine oxidase can produce ROS, a positive feedback loop in which mitochondria derived ROS activate xanthine oxidase, whose products further impair mitochondrial function, could develop. As such, mitochondria might fail to provide adequate adenosine triphosphate to satisfy the increased cardiac energy demand of rats with aortocaval

fistulae. Furthermore, the authors speculate that the increasing concentration of adenosine diphosphate might directly inhibit cardiac relaxation. Therefore, the authors suggest that xanthine oxidase inhibition improved mitochondrial respiration, which in turn improved cardiac function (Gladden et al., 2011). Interestingly, reversal of aortocaval fistulae results in significant improvement in haemodynamics, myocardial structure, and cardiac function (Hutchinson et al., 2011; Abassi et al., 2001a).

1.4. Rationale of this study

Congestive heart failure is a debilitating disease (review: Abassi et al., 2011). With its high morbidity and mortality, heart failure is a burden to healthcare resources, a burden to financial resources, and results in lost productivity (Public Health Agency of Canada, 2009). More than 500 000 Canadians suffer with heart failure, and approximately 50 000 additional patients are diagnosed annually (Ross et al., 2006). In 2005/2006 there were 54 333 hospitalizations due to heart failure in Canada (Public Health Agency of Canada, 2009). Given the increasing size of the elderly population, the frequency of hospitalization is expected to increase (Ross et al., 2006).

Rats with aortocaval fistulae display several traits of human heart failure, including: neurohormonal activation, haemodynamic abnormalities, and cardiac remodeling (review: Abassi et al., 2011; Hasenfuss, 1998). However, the hyperdynamic circulation might be used to model other physiological conditions such as: septic shock, anemia, portal hypertension, severe burns, and hyperthyroidism (Palmieri et al., 2004;

Anand et al., 1995; review: Williams et al., 2011; Hollenberg et al., 2004; Wattanasirichaigoon et al., 2000).

Systemic vascular flow (cardiac output – (fistula flow + bronchial flow)) is reduced in rats with aortocaval fistulae (Flaim et al., 1979). As such, regional perfusion is reduced in some vascular beds (Table 1; Bishara et al., 2008; Huang et al., 1992; Flaim et al., 1979). Similarly, heart failure is characterized by a decreased cardiac output, and results in decreased regional perfusion (review: Leier, 1992). For instance, renal blood flow is reduced in both human heart failure and this rat model (Bishara et al. 2008; review: Leier, 1992). Furthermore, hepatosplanchnic perfusion is reduced in human heart failure, this includes blood flow to: the stomach, the small intestine, the colon, the pancreas, and the liver (review: Leier, 1992; Parks and Jacobson, 1985). In rats with aortocaval fistulae, gastric and jejunal blood flows are decreased; whereas ileac, colonic, and hepatic blood flows are unaffected (Huang et al., 1992; Flaim et al., 1979).

The RAAS is activated in both human heart failure and rats with aortocaval fistulae (Karram et al., 2005; Abassi et al., 2001a; Willenbrock et al., 1999b; Pieruzzi et al., 1995; Ruzicka et al., 1993; review: Dube and Weber, 2011). Elevated plasma and tissue Ang II concentrations can decrease vascular conductance via direct vasoconstriction, sympathetic potentiation, and vascular hypertrophy (Li et al., 2008; review: Nguyen Dinh Cat and Touyz, 2011; Kobori et al., 2007). Vascular conductance is rapidly modulated by direct AT_1 receptor-mediated vasoconstriction and sympathetic potentiation (review: Touyz, 2005).

Table 1. Changes in regional blood flow due to aortocaval fistulae in rats.

*significantly different from sham-operated rats

Tissue		Time With Fistula	Change in Blood Flow (%)	Reference
Skeletal Muscle				
	Forelimb	1 hour	-55*	1
		1 day	-22	1
		1 week	-81*	1
		5 weeks	+60	1
	Hindlimb	1 hour	-50*	1
		1 day	-20	1
		1 week	+1060	1
		5 weeks	-25	1
	Gastrocnemius	1 day	-50*	2
		8 weeks	-50*	3
	Biceps	1 day	-14	2
		8 weeks	-38	3
	Triceps	1 day	-47*	2
		8 weeks	-56*	3
	Quadriceps	1 day	-44*	2
		8 weeks	-50*	3
	Latissimus dorsi	1 day	-13	2
		8 weeks	-58*	3
	Psoas	1 day	-62*	2
		8 weeks	-61*	3
	Quadriceps	1 day	-44*	2
		8 weeks	-50*	3
Skin				
	Dorsal	1 day	+77	2
	Limb	1 day	+32	2
Brain				
		1 hour	+19	1
		1 day	-12	1
		1 week	-61*	1
		5 weeks	+47	1
	Cerebellum	1 day	+84*	2
		8 weeks	-9	3

	Cerebrum	1 day	+71*	2
		8 weeks	-19	3
Heart				
		1 hour	+4	1
		1 day	+27	1
		1 week	+48	1
		5 weeks	-9	1
	Left Ventricle	1 day	-53*	2
	Right Ventricle	1 day	-56*	2
Intestine				
		1 hour	-33	1
		1 day	-9	1
		1 week	-63*	1
		5 weeks	-18	1
	Ileum	1 day	+120	2
		8 weeks	-20	3
	Jejunum	1 day	+50	2
		8 weeks	-21*	3
	Colon	1 day	+137*	2
		8 weeks	+9	3
Kidney				
		1 day	+29	2
		1 week	-50*	4
		1 week	-52*	5
		1 week	-66*	6
		4 weeks	-26	7
		8 weeks	-19	3
	Left	1 hour	-36*	1
		1 day	-15*	1
		1 week	-64*	1
		5 weeks	-15	1
	Right	1 hour	-24	1
		1 day	-16*	1
		1 week	-65*	1
		5 weeks	-24*	1
	Cortex	1 week	-30*	8
		1 week	-58*	9
	Medulla	1 week	-1	8
		1 week	-28	9

Liver				
		1 hour	+69*	1
		1 day	-77	1
		1 day	+140*	2
		1 week	-22	1
		5 weeks	+53	1
		8 weeks	-3	3
Spleen				
		1 hour	-39	1
		1 day	+27	1
		1 day	+4	2
		1 week	-53	1
		5 weeks	+5	1
		8 weeks	+9	3
Stomach				
		1 hour	-30	1
		1 day	-52*	1
		1 day	+8	2
		1 week	-45*	1
		5 weeks	-14	1
		8 weeks	-45*	3
Testicle				
		1 day	+49	2
		8 weeks	-31*	3
	Left	1 hour	-41*	1
		1 day	-23*	1
		1 week	-62*	1
		5 weeks	-8	1
	Right	1 hour	-50*	1
		1 day	-18*	1
		1 week	-54*	2
		5 weeks	+4	3

(1: Huang et al., 1992; 2: Flaim et al., 1980; 3: Flaim et al., 1979; 4: Brodsky et al., 1998; 5: Abassi et al., 1997; 6: Bishara et al., 2008; 7: Willenbrock et al., 1999a; 8: Abassi et al., 2001a; 9: Abassi et al., 1998a)

Acutely, Ang II causes vasoconstriction of the superior mesenteric and renal arteries of healthy rats, but not of the hindquarters (Table 2; Takemoto, 1999). However, acute ACE inhibition suggests that Ang II does not continuously influence the mesenteric and hindquarters blood flows and vascular resistances in healthy rats or in rats with myocardial infarction-induced heart failure (Nelissen-Vrancken et al., 1992). In contrast, ACE inhibition, AT_1 receptor antagonism, and AT_2 receptor antagonism suggest that the renal blood flow and vascular resistance of healthy rats and those with myocardial infarction or aortocaval fistula-induced heart failure are continuously regulated by Ang II (Brodsky et al., 1998; Mento et al., 1996; Nelissen-Vrancken et al., 1992). In humans, however, Ang II continuously regulates blood flow through the superior mesenteric artery, but not the renal artery, in healthy subjects and those with heart failure (Houghton et al., 1999; Ray-Chaudhuri et al., 1993). The increase in renal blood flow and decrease in renal vascular resistance with AT_1 receptor antagonism was greater in rats with aortocaval fistulae than in sham-operated rats, suggesting that Ang II has a greater influence over these parameters in rats with aortocaval fistulae than in sham-operated rats (Brodsky et al., 1998).

1.4.1. Hypothesis

Given that the RAAS is activated in rats with aortocaval fistulae, acute attenuation of the RAAS was expected to cause larger changes in regional blood flows and conductances of rats with aortocaval fistulae than sham-operated rats. AT_1 receptor

Table 2. Changes in regional blood flows and vascular resistances due to Ang II infusion, ACE inhibition, or Ang II receptor antagonism. ΔQ: change in blood flow; ΔR: change in resistance; HF: heart failure; MI: myocardial infarction; ACF: aortocaval fistula; NS: no significant change reported.

*significantly different from the baseline value

Blood Vessel	Species	Status	Ang II Infusion	ACE Inhibition		AT_1 Receptor Antagonism		AT_2 Receptor Antagonism		Reference
			ΔR (%)	ΔQ (%)	ΔR (%)	ΔQ (%)	ΔR (%)	ΔQ (%)	ΔR (%)	
Superior Mesenteric Artery	Rat	Healthy	+188*							1
				0	-3					2
		HF (MI)		+1	+3					2
	Human	Healthy		+69*	-37*					3
		HF		+23*		0				4
Hindquarters	Rat	Healthy	+14							1
				-5	+3					2
		HF (MI)		+2	-1					2
Renal Artery	Rat	Healthy	+239*							1
				+13*	-12*					2
						+22*	-24*			5
						+6	-18*	-4	-2	6
		HF (MI)		+10*	-11*					2
						+4	-18*	-4	-11*	6
		HF (ACF)				+35*	-33*			5
	Human	HF		NS		NS				4

(1: Takemoto, 1999; 2: Nelissen-Vrancken et al., 1992; 3: Ray-Chaudhuri et al., 1993; 4: Houghton et al., 1999; 5: Brodsky et al., 1998; 6: Mento et al., 1996)

antagonism should cause blood flows and conductances to increase, while AT_2 receptor antagonism should cause regional blood flows and conductances to decrease.

As systemic blood flow is decreased in rats with aortocaval fistulae, the increases in regional blood flows and conductances produced by AT_1 receptor antagonism were expected to be larger than the decreases in regional blood flows and conductances produced by AT_2 receptor antagonism. A similar result was expected in sham-operated rats, as the AT_1 receptor antagonist eprosartan had been shown to increase renal blood flow in rats, while renal blood flow was unchanged by the AT_2 receptor antagonist PD 123319 (Brodsky et al., 1998; Mento et al., 1996).

1.4.2. Objectives

To compare the changes in regional (mesenteric, renal, and hindquarter) blood flows and conductances produced by (a) the ACE inhibitor captopril, (b) the AT_1 receptor antagonist losartan, and (c) the AT_2 receptor antagonist PD 123319, between sham-operated and aortocaval fistulae rats.

To compare the changes in regional blood flows and conductances produced by the AT_1 receptor antagonist losartan to those produced by the AT_2 receptor antagonist PD 123319 in (a) sham-operated rats, and in (b) rats with aortocaval fistulae.

2. Materials and methods

All procedures on animals were carried out in accordance with the guidelines of the Canadian Council on Animal Care, with the approval of the Institutional Animal Care Committee of Memorial University of Newfoundland.

2.1. Establishment of aortocaval fistulae

Aortocaval fistulae were established similar to the method of Guo and Tabrizchi (2008). Male Sprague Dawley rats (5-6 weeks, 190-243 g) were anaesthetized with isoflurane (induction: 5% in 100% oxygen; maintenance: 1.5% in 100% oxygen with adjustments to maintain surgical anaesthesia). Heparin (0.25 mL, 100 units/mL, in normal saline; 0.9% sodium chloride in water) was injected subcutaneously prior to creating a midline abdominal incision. The abdominal aorta and inferior vena cava were exposed and dissected free from surrounding tissues inferior to the left renal vasculature and superior to the aortic bifurcation. Vascular clamps were placed at these locations to stop blood flow through the abdominal aorta and inferior vena cava.

In sham-operated rats, a 20G1 needle was advanced through the left aortic wall into the aortic lumen, approximately midway between the left renal vasculature and the aortic bifurcation, and then withdrawn. To establish aortocaval fistulae, the needle was further advanced through the right aortic and left vena caval walls into the vena caval lumen. In all rats, the puncture in the left aortic wall was closed with polypropylene suture (6-0, Ethicon, USA). Generally, the vascular clamps were removed within five minutes of placing them on the abdominal aorta and inferior vena cava. Fistula formation

was verified by: visible mixing of bright arterial blood and dark venous blood, or bright arterial blood flowing backwards into the iliolumbar veins upon compression of the inferior vena cava inferior to the left renal vasculature.

The abdominal muscle was closed with simple running sutures of chromic gut (3-0, Syneture, USA), and the skin was closed with horizontal mattress sutures of braided silk (3-0, Surgical Specialties Corporation, USA). Bupivacaine (1% in normal saline) was applied topically to the abdominal muscle and skin. Buprenorphine (0.2 mL, 0.5 mg/mL, in normal saline) was injected subcutaneously immediately following surgery and at twelve-hour intervals (0.15 mL, 0.5 mg/mL, in normal saline) for thirty-six hours. Ten percent of the rats with aortocaval fistulae died within five weeks of introduction of aortocaval fistulae.

2.2. Surgical preparation

Following a recovery period (5-7 weeks), experiments were conducted with surgical preparation similar to other studies (McClure et al., 2011; Guo and Tabrizchi, 2008). Rats (sham: 485 ± 5 g, n=38; fistula: 475 ± 5 g, n=32) were anaesthetized with isoflurane (induction: 5% in 100% oxygen; maintenance: 1.5% in 100% oxygen with adjustments to maintain surgical anaesthesia). An incision between the ventral right upper thigh and pelvis provided access to the right external iliac vein and artery, which were catheterized with polyethylene tubing (PE50; Becton Dickinson and Company, USA) for intravenous injection and blood pressure measurement, respectively. Both catheters were filled with heparinized normal saline (25 units/mL). Isoflurane administration was

stopped and thiobutabarbital sodium (80 mg/mL, in 160 μL 1 M sodium hydroxide and 840 μL normal saline) was periodically injected intravenously throughout surgery (sham-operated total: 95-139 mg/kg; aortocaval fistulae total: 81-137 mg/kg), as per lab protocol. An incision of the ventral neck provided access to the trachea between the submaxillary glands; the trachea was catheterized inferior to the isthmus of thyroid with a fourteen-gauge Teflon I.V. catheter (Abbott Hospitals, Inc., USA).

A midline abdominal incision from the xiphoid process to the bladder provided access to: the left renal artery, the abdominal aorta, the superior mesenteric artery, and the left common iliac artery. The arteries were dissected free from surrounding tissues and a transit-time perivascular flow probe (renal artery: MA1PRB; mesenteric artery: MA1.5PRB; abdominal aorta: MA3PSB; common iliac artery: 2SB2105; Transonic Systems Inc., USA) was placed on each blood vessel (Figure 3). The abdominal aorta flow probe was placed inferior to the left renal vasculature, superior to the remaining polypropylene suture. A thermometer was inserted into the rectum and the animal's temperature (35-38 °C) was maintained by adjusting heating lamps. The abdominal incision was covered with wet gauze before allowing the rat to stabilize for fifty minutes.

2.3. Continuous recording of haemodynamic parameters

Arterial blood flows and pressure were continuously recorded in AcqKnowledge 3.9.1.6, which simultaneously calculated heart rate from the variations in arterial pressure. Equipment setup was similar to that of Guo and Tabrizchi (2008). Specifically, arterial pressure was recorded from the right external iliac artery with a pressure

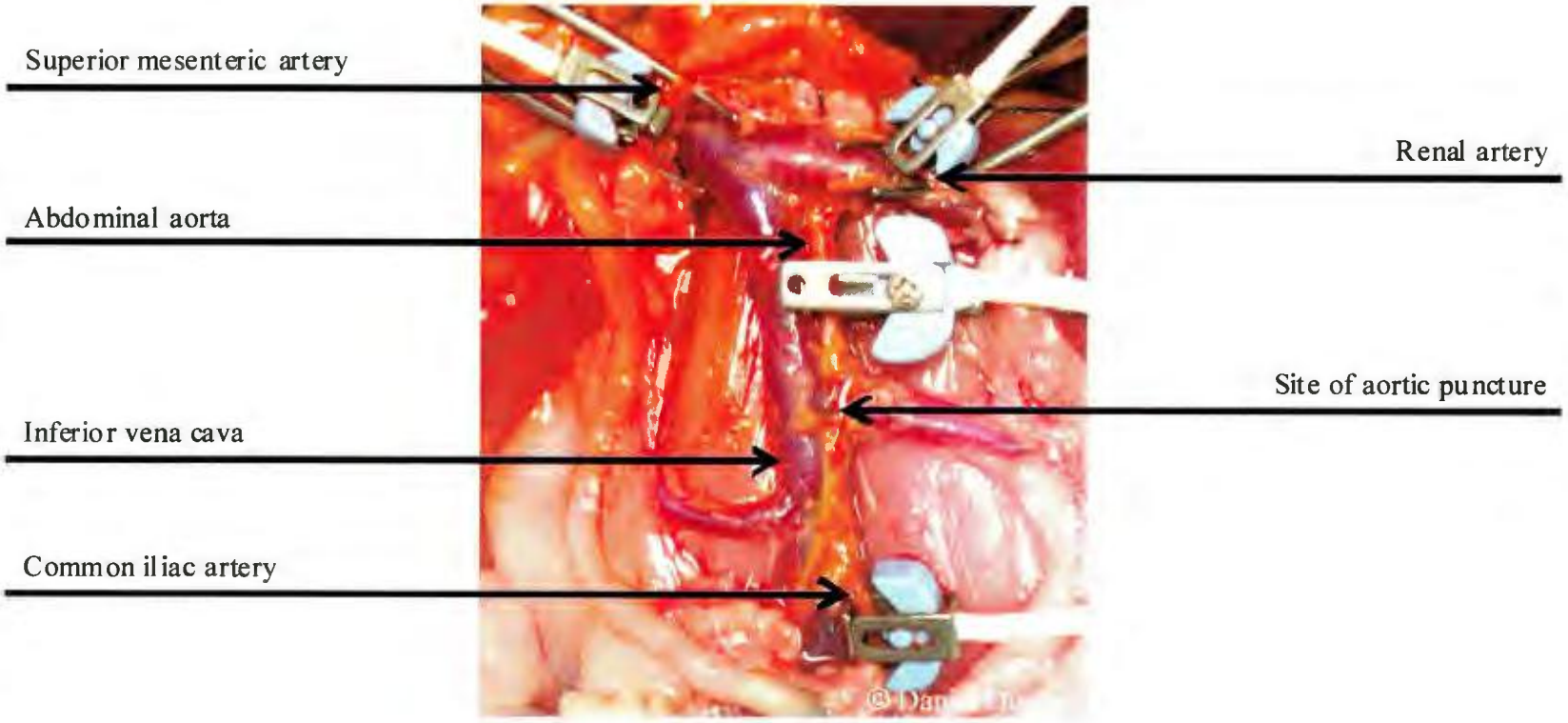

Figure 3. Flow probe placement and the site of aortic puncture.

Blood flow was measured in the left renal artery, the superior mesenteric artery, the abdominal aorta, and the left common iliac artery.

transducer (P23XL, Spectramed Statham, USA) and the signal was then amplified (DA 100A, Biopac Systems Inc., USA). Transit-time perivascular flowmeters (T106 and T403, Transonic Systems Inc., USA) recorded arterial blood flows (review: Tabrizchi and Pugsley, 2000). Both the amplified pressure signal and the flowmeter signals were passed through a universal interface module (UIM 100, Biopac Systems Inc., USA) into an acquisition unit (MP100, Biopac Systems Inc., USA), and the output signal was converted from analog to digital (USB1W, Biopac Systems Inc., USA) before registering in AcqKnowledge 3.9.1.6.

2.4. Experimental protocols

The aortocaval fistulae and sham-operated groups of rats were further divided into four treatment subgroups: captopril (an angiotensin-converting enzyme inhibitor), losartan (an AT_1 receptor antagonist), PD 123319 (an AT_2 receptor antagonist), and normal saline (the solvent in each of the other subgroups).

Following a ten-minute baseline recording, four doses of either: captopril (0.3, 1.0, 3.0, 10 mg/kg), losartan (2.5, 5.0, 10, 20 mg/kg), PD 123319 (0.1, 0.3, 1.0, 3.0 mg/kg), or normal saline (67, 200, 67, 200 µL/kg), were administered intravenously at approximately twenty-minute intervals (Figure 4; Tabrizchi and Lupichuk, 1995; Scheuer and Perrone, 1993; Stromberg et al., 1993). Normal saline injections were volume-matched to those of PD 123319, the largest volumes injected. Upon completion, rats were euthanized by intravenous injection of saturated potassium chloride (3 mL). The heart

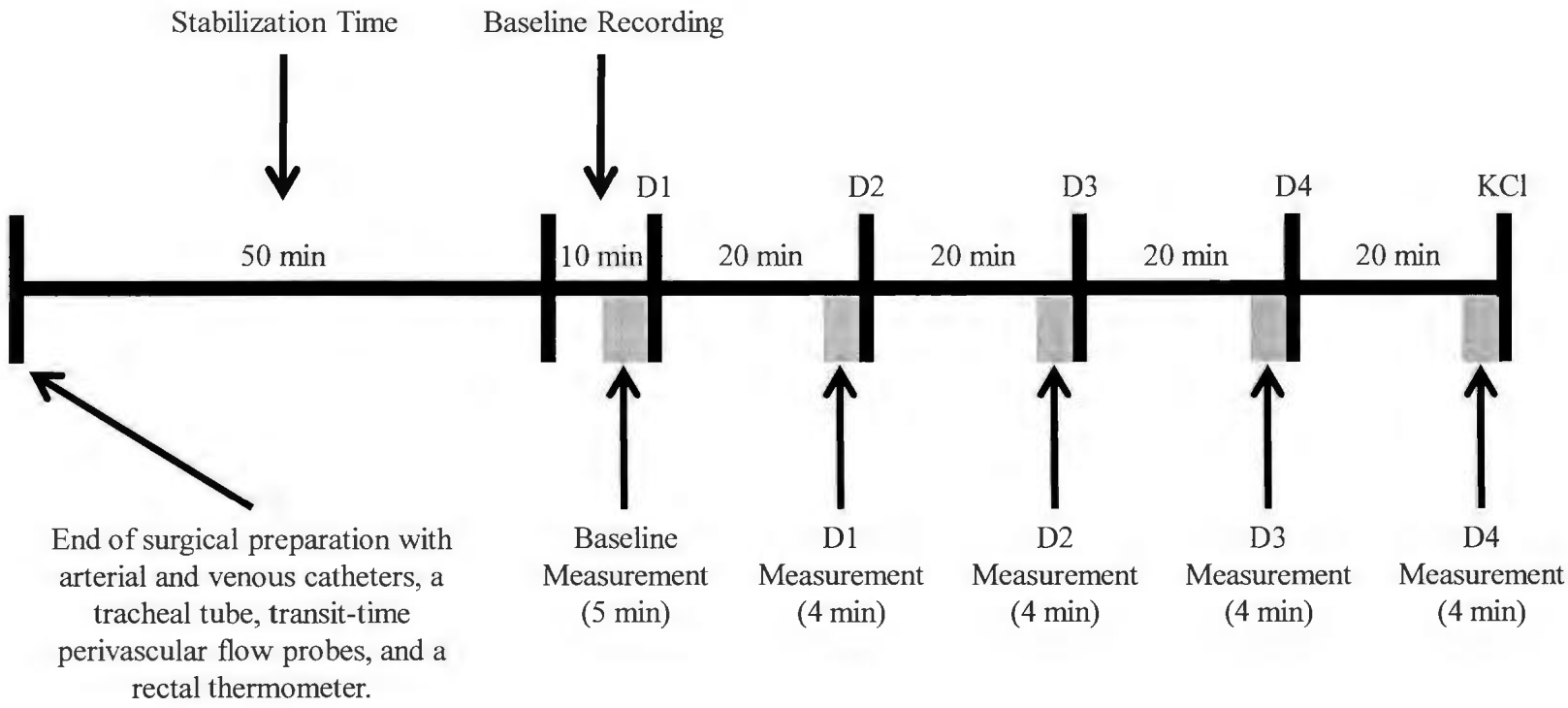

Figure 4. Timeline of experimental protocols.

(left ventricle and septum separated from the right ventricular wall) was weighed, dried for six hours (59-61 °C), and then weighed again.

2.5. Data measurements and calculations

Baseline values of continuously recorded blood flows, pressures, and calculated heart rates were measured as the average of the five minutes immediately preceding the first dose of treatment. Post-dose values of the same parameters were measured as the average of the four minutes immediately preceding the next dose of treatment, starting approximately sixteen minutes after administration of the preceding dose. Arterial conductance was calculated for each blood vessel (flow/mean arterial pressure measured in the external iliac artery; Lautt, 1988). Arterial pressure has been shown to vary between 5-6 mmHg in different arteries; therefore an inherent error is associated with each calculation of conductance, as the arterial pressure in each individual blood vessel was not recorded (Pang and Chan, 1985). However, it would not be feasible to measure blood flow and pressure in the same blood vessel at the same time, as the instrumentation required to measure one parameter may affect the other parameter.

To normalize the data, change from baseline was calculated for haemodynamic parameters by subtracting baseline values from post-dose values. Initial analysis by two-way analysis of variance with repeated measures indicated that the vehicle/time effects in rats with and without aortocaval fistulae differed with respect to mean arterial pressure and superior mesenteric blood flow. To allow for direct comparison of treatment effects between the groups, corrected changes from baseline were calculated for haemodynamic

parameters. In parallel, the mean change from baseline of the saline rats at each saline injection was subtracted from the respective change from baseline of each drug-treated rat within the same group (sham-operated and aortocaval fistulae rats).

All data are presented as a mean ± the standard error of the mean, and the number of animals (n). All graphs were prepared in SigmaPlot 8.0 (SPSS Inc., USA).

2.6. Statistical analyses

Baseline values of arterial flows, arterial conductances, mean arterial pressure and heart rate were compared between the sham-operated and the aortocaval fistulae groups of rats by unpaired two-tailed Student's t-tests. Similarly, ventricular weights were compared between sham-operated and aortocaval fistulae rats by unpaired two-tailed Student's t-tests. Rat weights (initial, weekly, experiment), were compared between the sham-operated and the aortocaval fistulae groups of rats by two-way analysis of variance with repeated measures. Baseline haemodynamic values were used to determine subgroup homogeneity within the sham-operated group of rats and within the aortocaval fistulae group of rats by one-way analysis of variance.

Change from baseline was used to compare the effects of captopril, losartan and PD 123319 to that of normal saline within the sham-operated group of rats and within the aortocaval fistulae group of rats, by two-way analysis of variance with repeated measures. Similarly, the change from baseline in haemodynamic parameters of both normal saline subgroups were compared by two-way analysis of variance with repeated measures.

Corrected change from baseline was used to compare treatment effects between the sham-operated and the aortocaval fistulae groups of rats, and between drug treatments within each group of rats, by two-way analysis of variance with repeated measures.

All statistical analyses were completed in SigmaStat 3.10.0 (Systat Software Inc., USA), $p<0.05$ was considered significant for all statistical analyses. When statistically significant differences were detected by analysis of variance, the Holm-Sidak method was used for correction of multiple comparisons testing.

2.7. Data exclusion

Seventy experimental protocols were successfully completed; however, six of these were excluded from the final analysis for the following reasons: (1) high baseline renal blood flow in a sham-operated rat (excluded: 17.2 mL/min, remaining: 8.3 ± 0.4 mL/min, n=34), (2) low baseline mesenteric blood flow in a sham-operated rat (excluded: 1.1 mL/min, remaining: 10.0 ± 0.5 mL/min, n=34), (3) low baseline mean arterial pressure in a sham-operated rat (excluded: 50 mmHg, remaining: 95 ± 3 mmHg, n=34), (4) large increase in mesenteric blood flow upon administration of captopril in a rat with aortocaval fistula (excluded: +13.3 mL/min, remaining: +1.6 ± 1.5 mL/min, n=6), (5) large decrease in mean arterial pressure upon administration of normal saline in a rat with aortocaval fistula (excluded: -36 mmHg, remaining: 0 ± 4 mmHg, n=7), and (6) large increase in mean arterial pressure upon administration of normal saline in a sham-operated rat (excluded: +25 mmHg, remaining: -12 ± 3 mmHg, n=10).

2.8. Fine chemicals

Isoflurane was purchased from CDMV (Canada). Heparin sodium was obtained from SoloPak Laboratories Inc. (USA) and Sigma-Aldrich Inc. (USA). Sigma-Aldrich Inc. (USA) also supplied bupivacaine hydrochloride, buprenorphine hydrochloride, captopril, and thiobutabarbital sodium. PD 123319 ditrifluoroacetate was purchased from Tocris Bioscience (UK), and losartan potassium was donated by DuPont Merck Pharmaceutical Company (USA).

3. Results

3.1. Body weights and ventricular weights

Sham-operated and aortocaval fistulae rats had similar weekly body weights following fistula formation, and similar body weights at the time of experimentation. The left ventricle and septum and right ventricle of rats with aortocaval fistulae were significantly heavier than those of sham-operated rats when either the wet or the dry weights were compared (Table 3). However, the ratios of left ventricle and septum to right ventricle were similar between rats with and without aortocaval fistulae when calculated using either wet or dry weights.

3.2. Baseline haemodynamic values

Sham-operated and aortocaval fistulae rats were similar with respect to baseline mean arterial pressure (MAP) and heart rate (HR; Figure 5AB). However, aortic blood flow and conductance (ABF, AC) were significantly higher, while the mesenteric and renal blood flows and conductances (MBF, RBF, MC, RC) were significantly lower, in rats with aortocaval fistulae compared to sham-operated rats (Figure 6ABCD). Iliac blood flow and conductance (IBF) was similar in both groups.

3.3. Baseline haemodynamic values by treatment group

Recorded and calculated baseline values were analyzed to ensure similar values amongst the treatment groups of sham-operated rats, and amongst the treatment groups of rats with aortocaval fistulae (Tables 4 and 5). Baseline values of treatment groups were

Table 3. Ventricular hypertrophy in rats with aortocaval fistulae.
Weights (g) and ratios of wet or dry ventricles of sham-operated and aortocaval fistulae rats. Values are mean ± SEM. LV+S: left ventricle and septum; RV: right ventricle.

*significantly different from the respective sham value

Group	n	Wet			Dry		
		LV+S	RV	LV+S/RV ratio	LV+S	RV	LV+S/RV ratio
Sham	34	0.81 ± 0.01	0.28 ± 0.01	2.96 ± 0.06	0.29 ± 0.01	0.08 ± 0.01	3.83 ± 0.13
Fistula	30	1.16 ± 0.03*	0.52 ± 0.07*	2.66 ± 0.18	0.49 ± 0.03*	0.13 ± 0.01*	3.91 ± 0.19

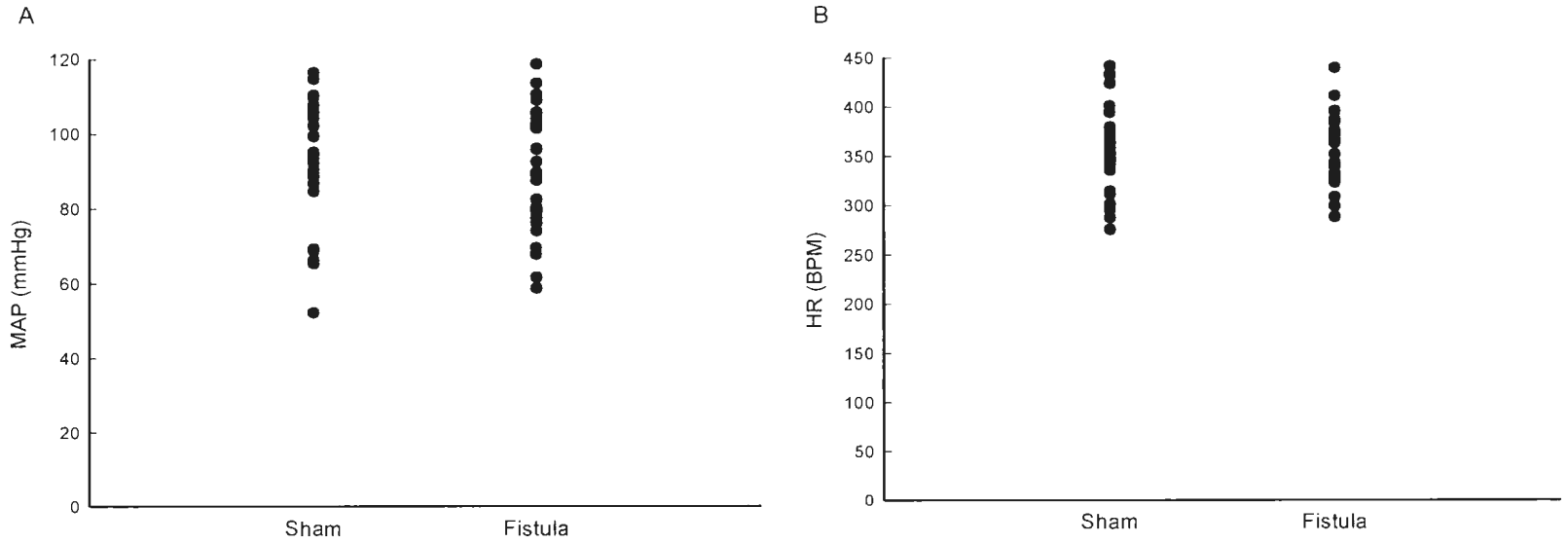

Figure 5. Baseline mean arterial pressures and heart rates of anaesthetized rats five weeks after sham or fistula surgery. Sham-operated rats: MAP=95±3 mmHg, HR=362±8 BPM, n=34; rats with aortocaval fistulae: MAP=88±3 mmHg, HR=362±7, n=30. MAP: mean arterial pressure; HR: heart rate.

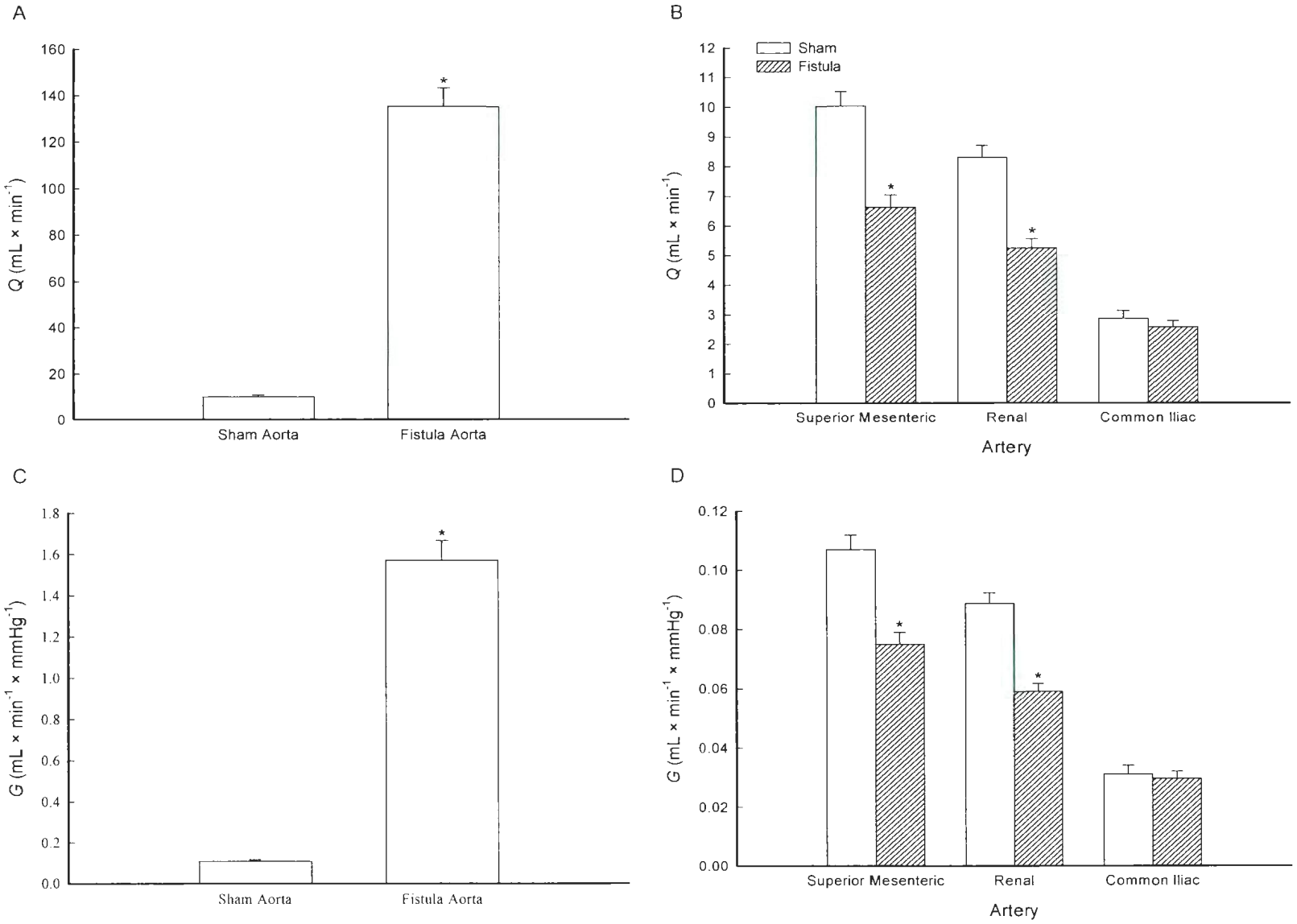
A
160
140
120
100
80
60
40
20
0
Q (mL × min-1)
*
Sham Aorta
Fistula Aorta
B
Sham
Fistula
12
11
10
9
8
7
6
5
4
3
2
1
0
Q (mL × min-1)
*
*
Superior Mesenteric
Renal
Common Iliac
Artery
C
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
G (mL × min-1 × mmHg-1)
*
Sham Aorta
Fistula Aorta
D
0.12
0.10
0.08
0.06
0.04
0.02
0.00
G (mL × min-1 × mmHg-1)
*
*
Superior Mesenteric
Renal
Common Iliac
Artery

Figure 6. Baseline blood flows and conductances of anaesthetized rats five weeks after sham or fistula surgery.

All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM.

Sham-operated rats, n=34; rats with aortocaval fistulae, n=30. *Q*: blood flow; *G*: vascular conductance.

*significantly different from the sham group, $p<0.05$

Table 4. Baseline heart rate, mean arterial pressure, and blood flows in rats with and without aortocaval fistulae. Values are mean ± SEM. HR: heart rate; MAP: mean arterial pressure.

*significantly different from sham-operated captopril and saline, $p<0.05$

Group	Subgroup	n	HR (BPM)	MAP (external iliac artery; mmHg)	Blood Flows ($mL \times min^{-1}$)			
					Aortic	Mesenteric	Renal	Iliac
Sham-Operated	Saline	10	347 ± 12	101 ± 2	10.4 ± 1.6	12.4 ± 0.7	9.2 ± 0.8	2.3 ± 0.4
	Captopril	7	372 ± 16	98 ± 3	10.9 ± 1.3	11.9 ± 1.1	9.4 ± 0.7	2.8 ± 0.4
	Losartan	9	374 ± 13	97 ± 6	9.7 ± 1.3	8.0 ± 0.5*	8.0 ± 0.6	3.4 ± 0.8
	PD 123319	8	360 ± 20	81 ± 9	9.5 ± 1.4	7.8 ± 0.4*	6.6 ± 0.8	3.0 ± 0.4
Aortocaval Fistula	Saline	7	377 ± 17	86 ± 7	119 ± 14	7.2 ± 1.1	5.3 ± 0.9	2.8 ± 0.4
	Captopril	6	348 ± 21	83 ± 4	155 ± 11	5.9 ± 0.7	4.6 ± 0.4	3.2 ± 0.6
	Losartan	9	356 ± 12	97 ± 6	139 ± 20	7.3 ± 0.8	6.1 ± 0.6	2.3 ± 0.4
	PD 123319	8	368 ± 8	81 ± 9	130 ± 12	6.1 ± 0.6	4.8 ± 0.5	2.0 ± 0.3

Table 5. Baseline regional vascular conductances in rats with and without aortocaval fistulae.
All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM.

*significantly different from sham-operated captopril and saline, $p<0.05$

Group	Subgroup	n	Conductance (mL × min^{-1} × mmHg^{-1})			
			Aortic	Mesenteric	Renal	Iliac
Sham-Operated	Saline	10	0.10 ± 0.02	0.12 ± 0.01	0.09 ± 0.01	0.02 ± 0.00
	Captopril	7	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.03 ± 0.01
	Losartan	9	0.10 ± 0.02	0.08 ± 0.01*	0.08 ± 0.01	0.03 ± 0.01
	PD 123319	8	0.12 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.04 ± 0.01
Aortocaval Fistula	Saline	7	1.4 ± 0.2	0.08 ± 0.01	0.06 ± 0.01	0.03 ± 0.01
	Captopril	6	1.9 ± 0.1	0.07 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
	Losartan	9	1.5 ± 0.2	0.08 ± 0.01	0.06 ± 0.00	0.03 ± 0.00
	PD 123319	8	1.6 ± 0.1	0.07 ± 0.01	0.06 ± 0.01	0.02 ± 0.00

not compared between sham-operated rats and rats with aortocaval fistulae because treatment effects were compared as changes from baseline. The MBF of the captopril and normal saline treatment groups of sham-operated rats were significantly greater than those of the losartan and PD 123319 treatment groups. Furthermore, the MC of the captopril and normal saline treatment groups were significantly greater than that of the losartan treatment group. No statistical differences in baseline values were detected amongst the treatment groups of rats with aortocaval fistulae.

3.4. Comparison of captopril, losartan, and PD 123319 to normal saline in sham-operated rats

PD 123319 significantly increased mean arterial pressure (ΔMAP), whereas the reductions by captopril and losartan were similar to that of the normal saline group (Figure 7A). The changes in heart rate (ΔHR) produced by captopril, losartan, and PD 123319 were similar to that of the normal saline group, although increasing doses of captopril tended to decrease ΔHR (Figure 7B).

The changes in: aortic blood flow (ΔABF), mesenteric blood flow (ΔMBF), renal blood flow (ΔRBF), and iliac blood flow (ΔIBF) produced by captopril, losartan, and PD 123319 were similar to those of the normal saline group (Figure 8ABCD). However, captopril, losartan, and PD 123319 tended to increase ΔMBF, whereas a time-dependent decrease in ΔMBF was observed in the normal saline group.

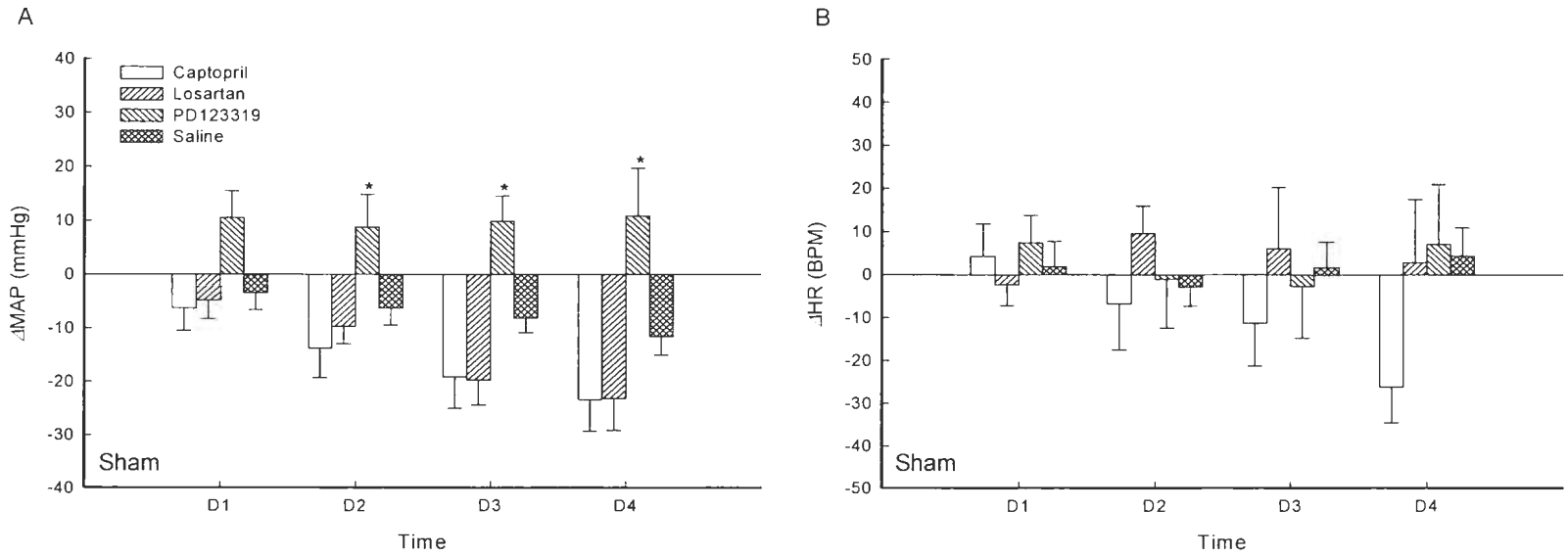

Figure 7. Changes in mean arterial pressure and heart rate due to drug treatments in sham-operated rats.

Changes in mean arterial pressure and heart rate of anaesthetized rats were compared to the respective time-effects of saline administration. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg, n=7; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg, n=9; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg, n=8, saline: 67, 200, 67, 200 µL/kg, n=10. SO: sham-operated; ΔMAP: change in mean arterial pressure; ΔHR: change in heart rate; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

*significantly different from the saline group, $p<0.05$

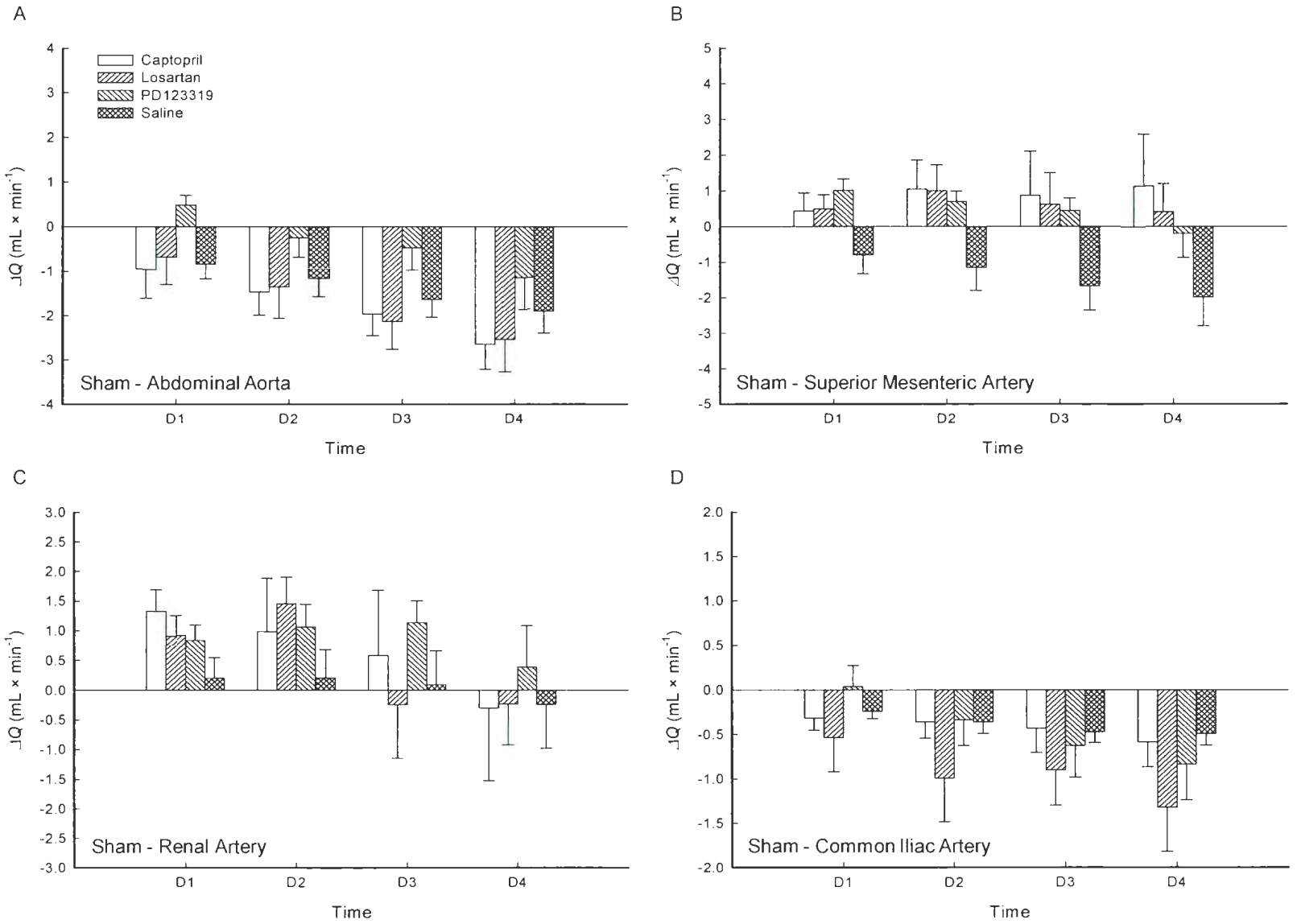
A
Captopril
Losartan
PD123319
Saline
ΔQ (mL × min-1)
Sham - Abdominal Aorta
D1
D2
D3
D4
Time
B
ΔQ (mL × min-1)
Sham - Superior Mesenteric Artery
D1
D2
D3
D4
Time
C
ΔQ (mL × min-1)
Sham - Renal Artery
D1
D2
D3
D4
Time
D
ΔQ (mL × min-1)
Sham - Common Iliac Artery
D1
D2
D3
D4
Time

Figure 8. Changes in blood flows due to drug treatments in sham-operated rats.

Changes in blood flows of anaesthetized rats were compared to the respective time-effects of saline administration. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg, n=7; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg, n=9; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg, n=8, saline: 67, 200, 67, 200 µL/kg, n=10. SO: sham-operated; ΔQ: change in blood flow; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

A time-dependent decrease in aortic conductance (ΔAC) was observed in the normal saline group (Figure 9A). Captopril and losartan suppressed the time-dependent decrease, whereas PD 123319 augmented the decrease. However, the ΔAC produced by captopril, losartan, and PD 123319 were similar to that of the normal saline group. In contrast, captopril and losartan significantly increased mesenteric conductance (ΔMC) compared to the normal saline group (Figure 9B). The decrease in ΔMC produced by PD 123319 was not significantly different from the normal saline group. Additionally, captopril and losartan caused significant increases in renal conductance (ΔRC) compared to the normal saline group (Figure 9C). In contrast, PD 123319 caused a significant decrease in ΔRC. PD 123319 significantly decreased iliac conductance (ΔIC) compared to normal saline, while captopril and losartan had no effect on ΔIC (Figure 9D).

3.5. Comparison of captopril, losartan, and PD 123319 to normal saline in rats with aortocaval fistulae

Captopril and losartan had significant dose-dependent hypotensive effects when compared to the normal saline group (Figure 10A). In contrast, PD 123319 had no significant effect on ΔMAP. The ΔHR were similar between the captopril, losartan, and PD 123319 groups and the normal saline group (Figure 10B).

Captopril and losartan caused significant dose-dependent decreases in ΔABF compared to the normal saline group, whereas the decrease produced by PD 123319 was not significant (Figure 11A). In contrast, losartan caused a significant dose-dependent

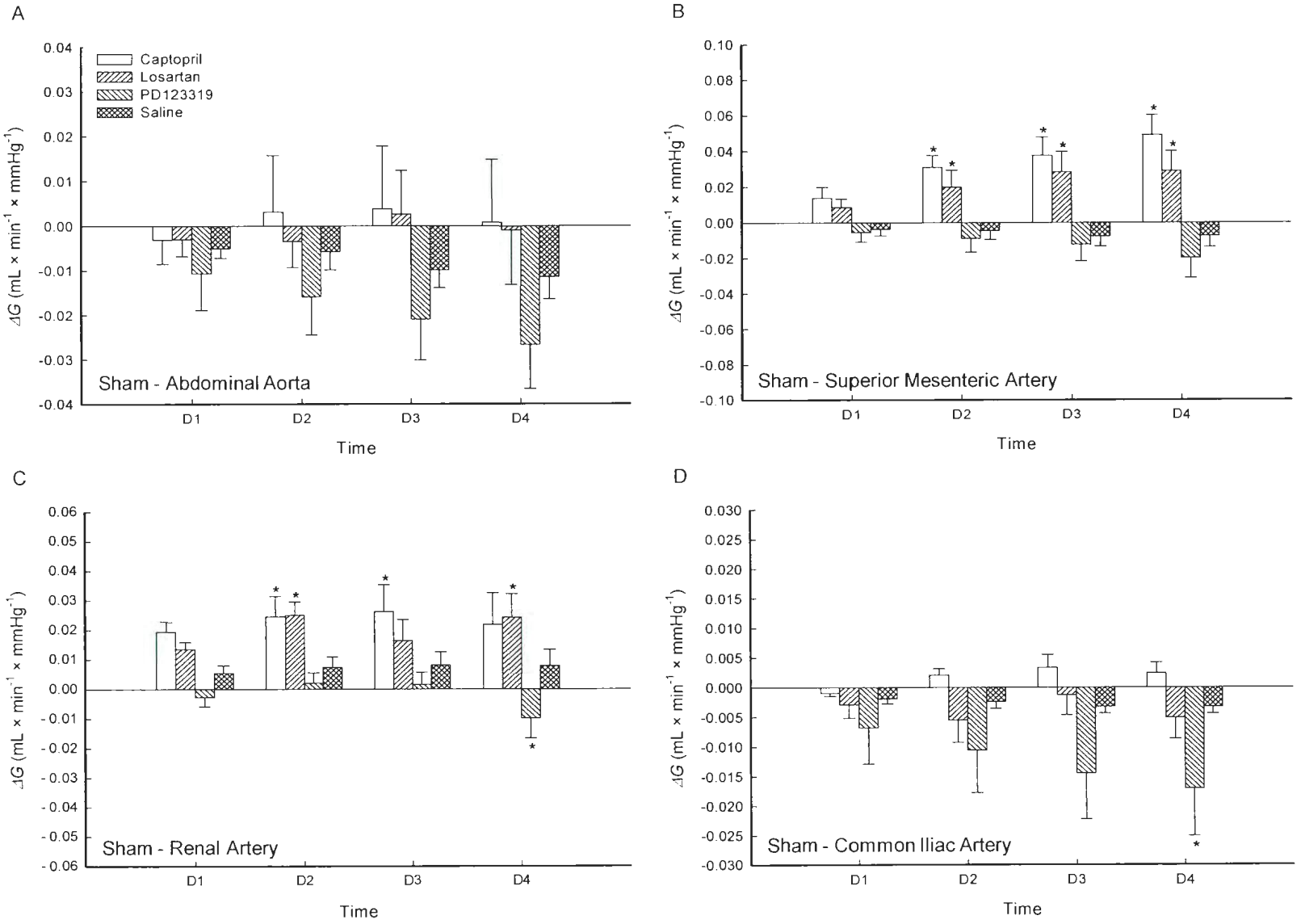
A
Captopril
Losartan
PD123319
Saline
ΔG (mL × min-1 × mmHg-1)
Sham - Abdominal Aorta
D1
D2
D3
D4
Time
B
Sham - Superior Mesenteric Artery
C
Sham - Renal Artery
D
Sham - Common Iliac Artery

Figure 9. Changes in vascular conductances due to drug treatments in sham-operated rats.

Changes in vascular conductances of anaesthetized rats were compared to the respective time-effects of saline administration. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg, n=7; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg, n=9; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg, n=8, saline: 67, 200, 67, 200 μL/kg, n=10. SO: sham-operated; ΔG: change in vascular conductance; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

*significantly different from the saline group, $p<0.05$

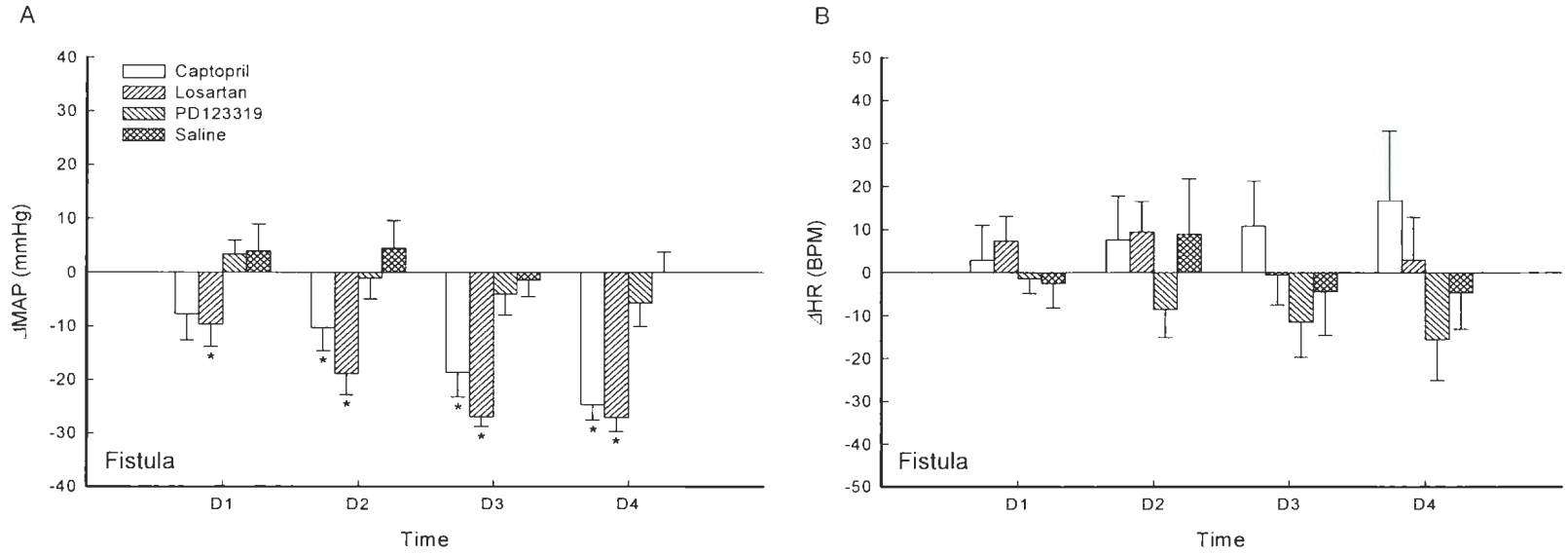

Figure 10. Changes in mean arterial pressure and heart rate due to drug treatments in rats with aortocaval fistulae.

Changes in mean arterial pressure and heart rate of anaesthetized rats were compared to the respective time-effects of saline administration. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg, n=6; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg, n=9; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg, n=8, saline: 67, 200, 67, 200 µL/kg, n=7. ACF: aortocaval fistulae; *Δ*MAP: change in mean arterial pressure; *Δ*HR: change in heart rate; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

*significantly different from the saline group, $p<0.05$

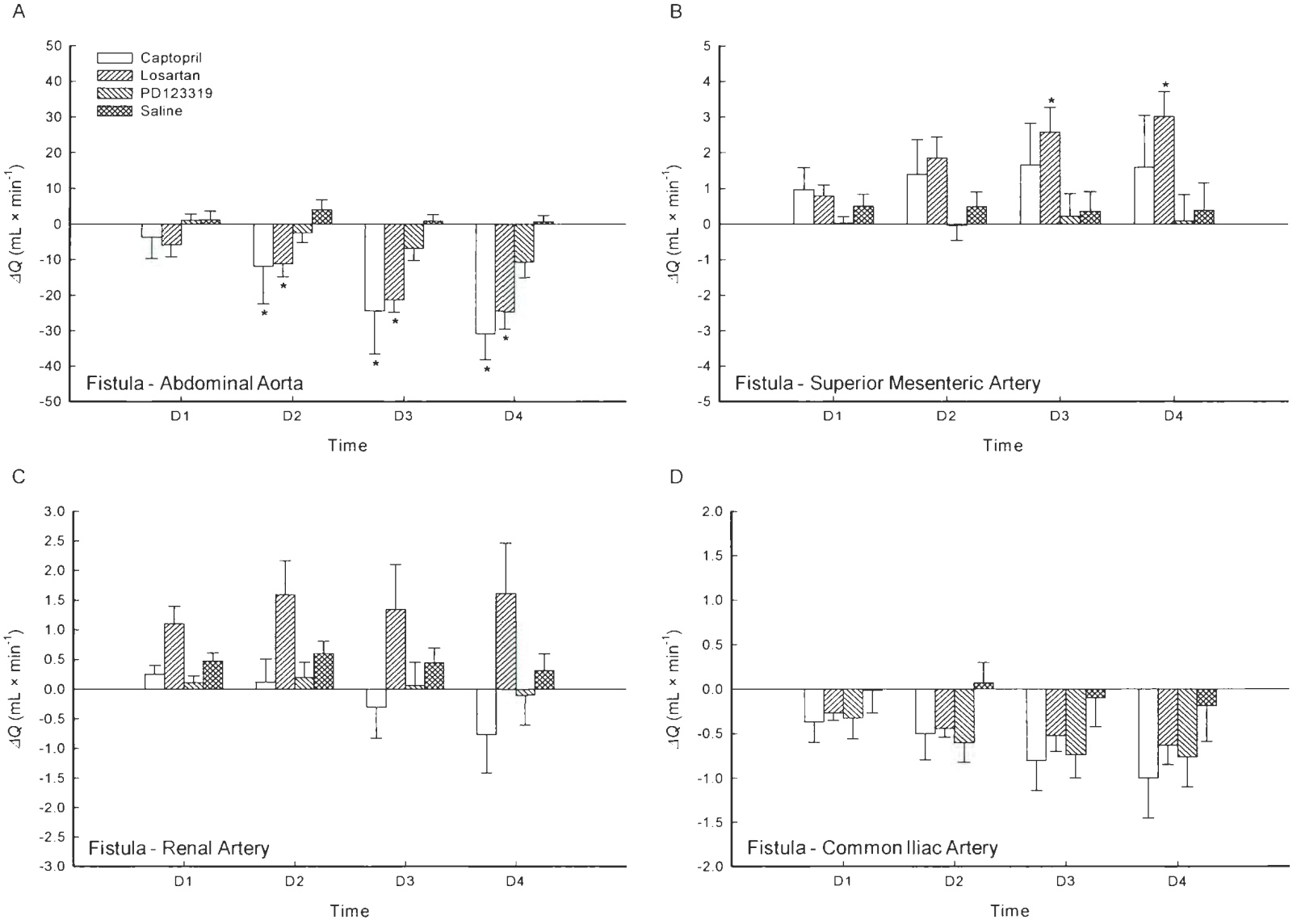
A
Captopril
Losartan
PD123319
Saline
ΔQ (mL × min⁻¹)
Fistula - Abdominal Aorta
D1
D2
D3
D4
Time
B
ΔQ (mL × min⁻¹)
Fistula - Superior Mesenteric Artery
D1
D2
D3
D4
Time
C
ΔQ (mL × min⁻¹)
Fistula - Renal Artery
D1
D2
D3
D4
Time
D
ΔQ (mL × min⁻¹)
Fistula - Common Iliac Artery
D1
D2
D3
D4
Time

Figure 11. Changes in blood flows due to drug treatments in rats with aortocaval fistulae.

Changes in blood flows of anaesthetized rats were compared to the respective time-effects of saline administration. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg, n=6; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg, n=9; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg, n=8, saline: 67, 200, 67, 200 µL/kg, n=7. ACF: aortocaval fistulae; ΔQ: change in blood flow; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

*significantly different from the saline group, $p<0.05$

increase in ΔMBF, while the increases produced by captopril and PD 123319 were not significantly different from the normal saline group (Figure 11B). The ΔRBF and ΔIBF produced by captopril, losartan, and PD 123319 were not significantly different from those of the normal saline group (Figure 11CD).

Losartan caused a significant dose-dependent increase in ΔAC compared to the normal saline group (Figure 12A). The increase in ΔAC produced by captopril and the variable effect of PD 123319 were not significantly different from the normal saline group. Captopril and losartan caused significant dose-dependent increases in ΔMC compared to the normal saline group, while PD 123319 had little effect (Figure 12B). Losartan caused a significant dose-dependent increase in ΔRC compared to the normal saline group, while neither captopril nor PD 123319 produced a significant effect (Figure 12C). ΔIC was not significantly affected by captopril, losartan, or PD 123319, when compared to the normal saline group (Figure 12D).

3.6. Comparison of the time effects in sham-operated and aortocaval fistulae rats

The time-dependent hypotensive effect of normal saline in sham-operated rats was significantly different from the variable ΔMAP of rats with aortocaval fistulae (Figure 13A). The ΔHR of both groups were similar (Figure 13B).

The ΔABF of both normal saline-treated groups of rats were not significantly different (Figure 14A). In contrast, the increase in ΔMBF in rats with aortocaval fistulae was significantly different from the time-dependent decrease in sham-operated rats (Figure 14B). The normal saline-treated groups of rats had similar ΔRBF and ΔIBF

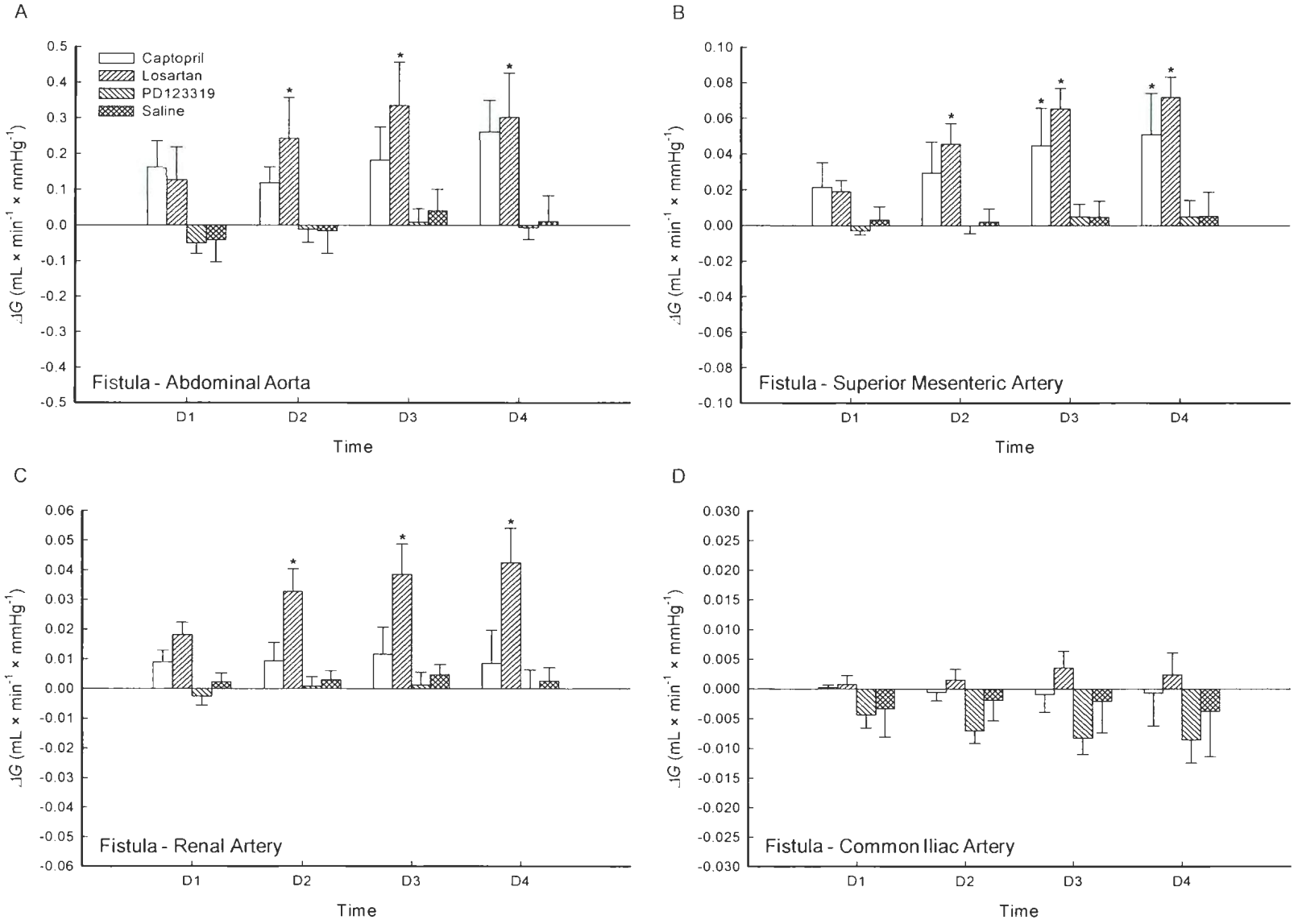
A
Captopril
Losartan
PD123319
Saline
ΔG (mL × min⁻¹ × mmHg⁻¹)
Fistula - Abdominal Aorta
D1
D2
D3
D4
Time
B
ΔG (mL × min⁻¹ × mmHg⁻¹)
Fistula - Superior Mesenteric Artery
Time
C
ΔG (mL × min⁻¹ × mmHg⁻¹)
Fistula - Renal Artery
Time
D
ΔG (mL × min⁻¹ × mmHg⁻¹)
Fistula - Common Iliac Artery
Time

Figure 12. Changes in vascular conductances due to drug treatments in rats with aortocaval fistulae.

Changes in regional vascular conductances of anaesthetized rats were compared to the respective time-effects of saline administration. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg, n=6; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg, n=9; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg, n=8, Saline: 67, 200, 67, 200 µL/kg, n=7. ACF: aortocaval fistulae; ΔG: change in vascular conductance; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

*significantly different from the saline group, $p<0.05$

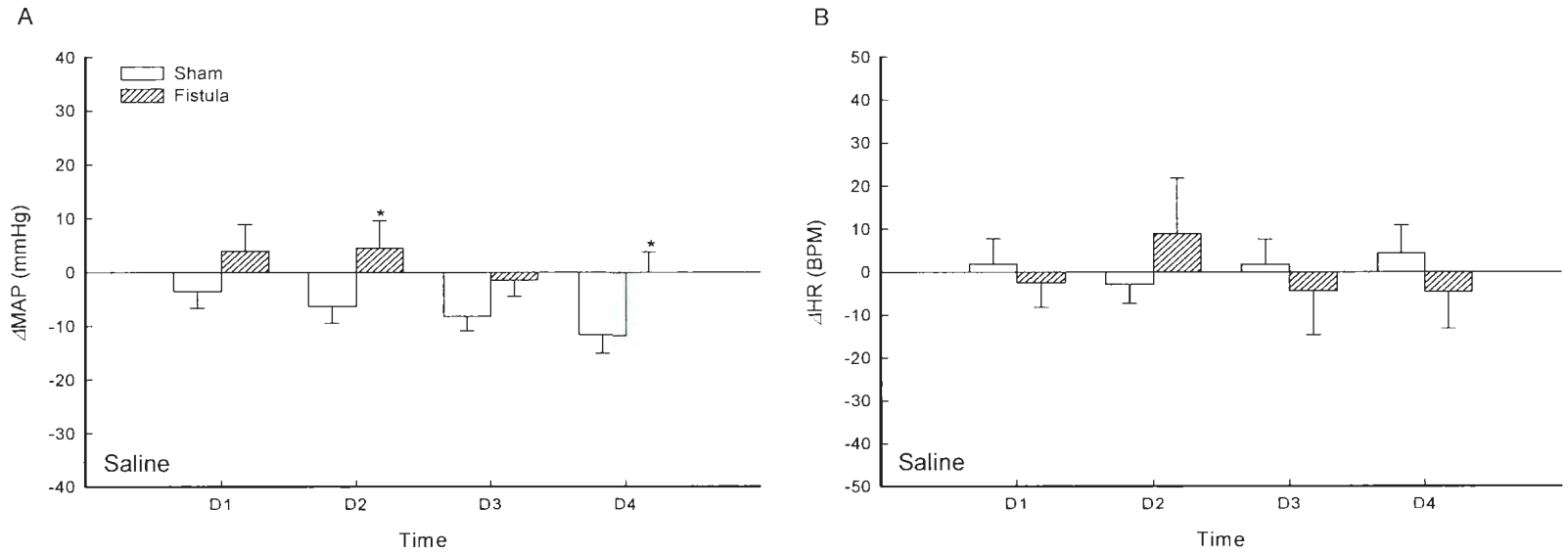

Figure 13. Changes in mean arterial pressure and heart rate due to saline treatment in rats with and without aortocaval fistulae. Volumes equivalent to the largest drug injections were administered to anaesthetized rats every twenty minutes following the control measurement to which subsequent measurements were normalized. Values are mean ± SEM. Saline: 67, 200, 67, 200 μL/kg; sham-operated rats: n=10; rats with aortocaval fistulae: n=7. *Δ*MAP: change in mean arterial pressure; *Δ*HR: change in heart rate; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

*significantly different from the sham-operated group, $p<0.05$

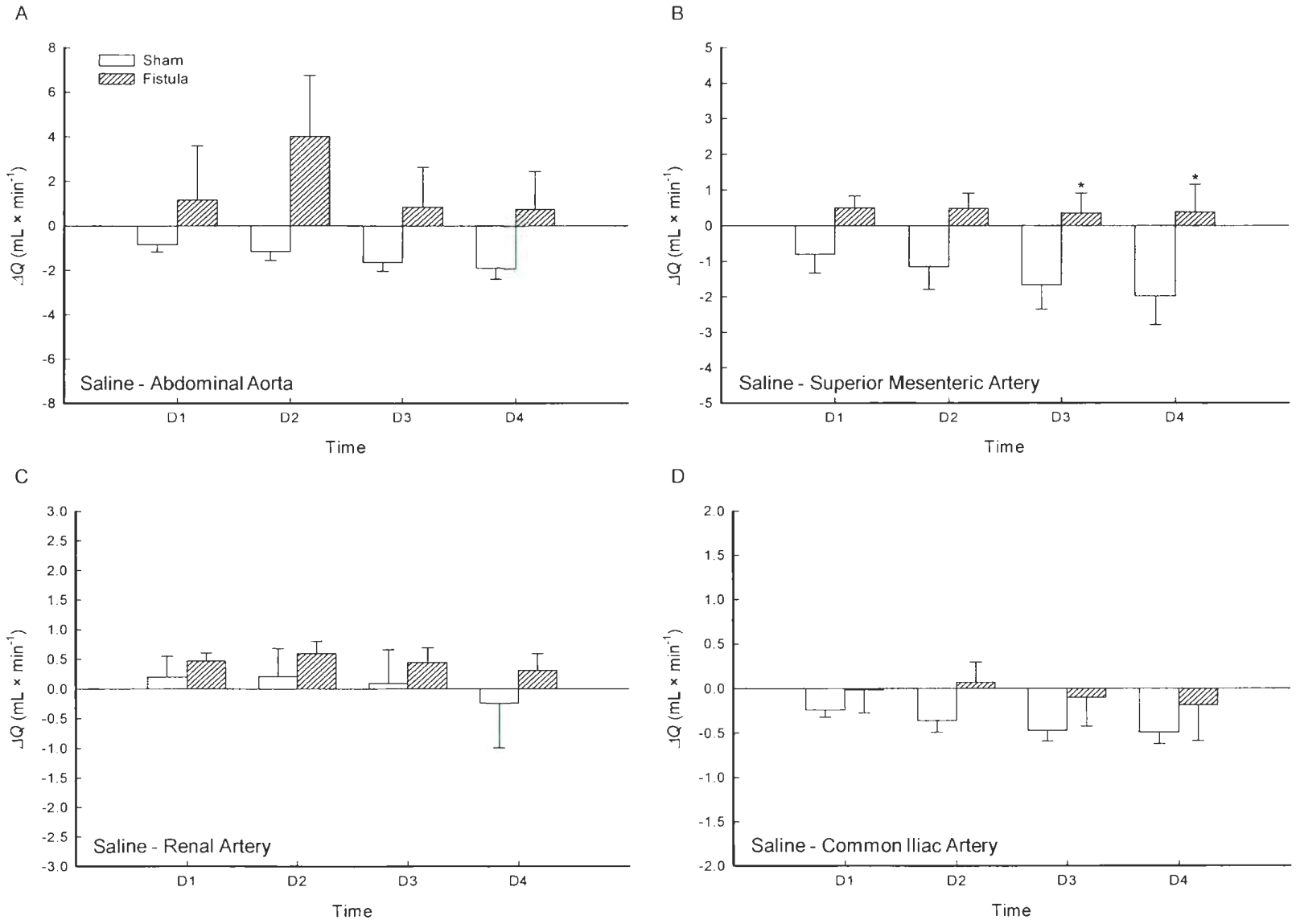
A
Sham
Fistula
ΔQ (mL × min⁻¹)
Saline - Abdominal Aorta
D1
D2
D3
D4
Time
B
ΔQ (mL × min⁻¹)
Saline - Superior Mesenteric Artery
*
*
D1
D2
D3
D4
Time
C
ΔQ (mL × min⁻¹)
Saline - Renal Artery
D1
D2
D3
D4
Time
D
ΔQ (mL × min⁻¹)
Saline - Common Iliac Artery
D1
D2
D3
D4
Time

Figure 14. Changes in blood flows due to saline treatment in rats with and without aortocaval fistulae.

Volumes equivalent to the largest drug injections were administered to anaesthetized rats every twenty minutes following the control measurement to which subsequent measurements were normalized. Values are mean ± SEM. Saline: 67, 200, 67, 200 μL/kg; sham-operated rats: n=10; rats with aortocaval fistulae: n=7. ΔQ: change in blood flow; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

*significantly different from the sham-operated group, $p<0.05$

(Figure 14CD). Also, both groups of normal saline-treated rats experienced similar changes in vascular conductances (ΔAC, ΔMC, ΔRC, and ΔIC; Figure 15ABCD).

3.7. Comparison of the effects of captopril, losartan, and PD 123319 on mean arterial pressure and heart rate

The dose-dependent hypotensive effects of captopril and losartan were significantly different from the hypertensive effect of PD 123319 in sham-operated rats (Figure 16A). Similarly, in rats with aortocaval fistulae, the hypotensive effects of captopril and losartan were significantly greater that the decrease in corrected mean arterial pressure (cΔMAP) caused by PD 123319 (Figure 16B). When compared between the two groups, the hypotensive effect of losartan was significantly greater in rats with aortocaval fistulae than in sham-operated rats. Similarly, the hypotensive effect or PD 123319 in rats with aortocaval fistulae was significantly different from the pressor effect observed in sham-operated rats.

The change in corrected heart rate (cΔHR) produced by captopril, losartan, and PD 123319 were similar in sham-operated rats and in rats with aortocaval fistulae (Figure 16CD). However, the increase in cΔHR of rats with aortocaval fistulae treated with captopril was significantly different from the dose-dependent decrease in cΔHR observed in sham-operated rats.

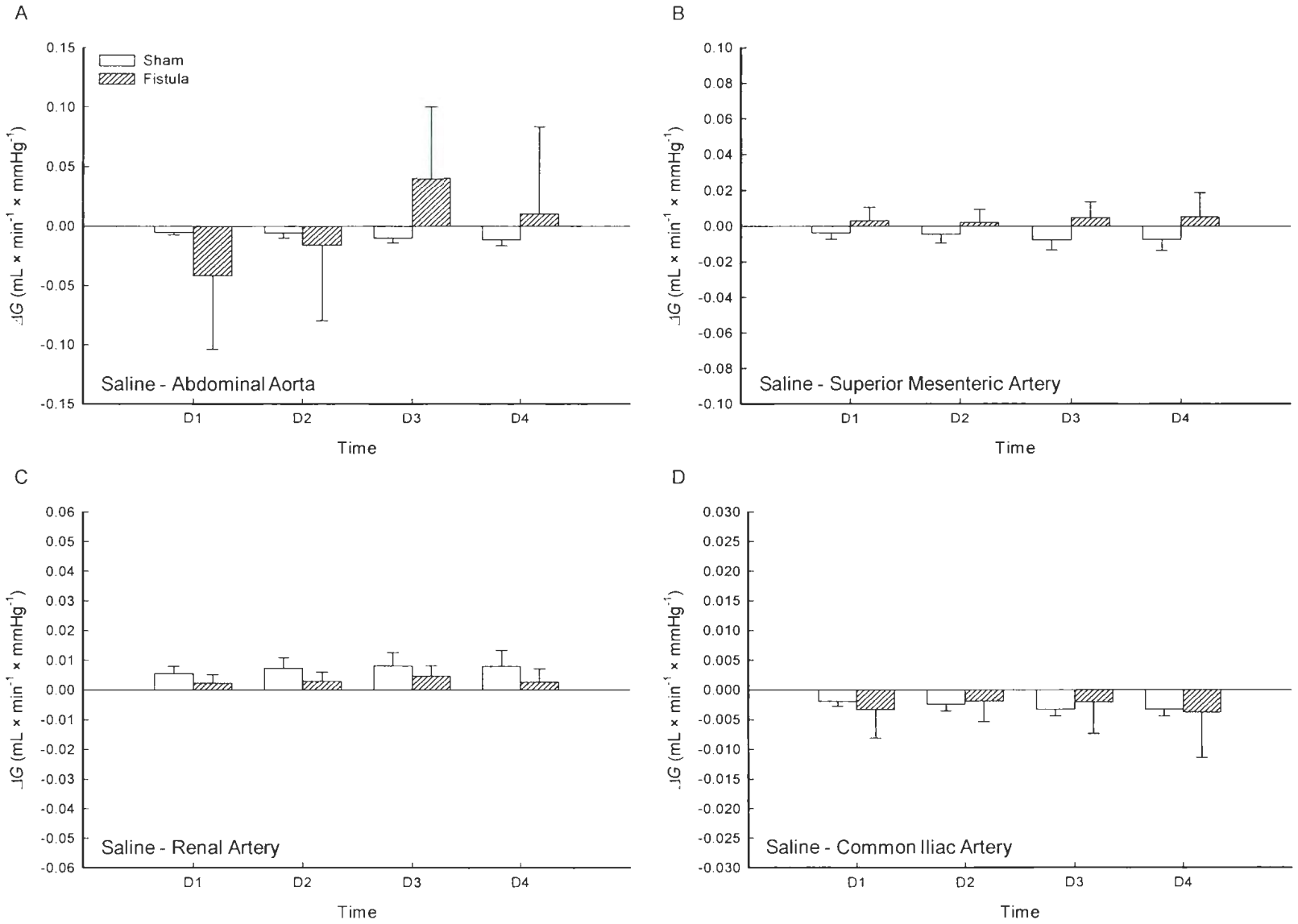
A
Sham
Fistula
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
ΔG (mL × min-1 × mmHg-1)
Saline - Abdominal Aorta
D1
D2
D3
D4
Time
B
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
ΔG (mL × min-1 × mmHg-1)
Saline - Superior Mesenteric Artery
D1
D2
D3
D4
Time
C
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
ΔG (mL × min-1 × mmHg-1)
Saline - Renal Artery
D1
D2
D3
D4
Time
D
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
-0.020
-0.025
-0.030
ΔG (mL × min-1 × mmHg-1)
Saline - Common Iliac Artery
D1
D2
D3
D4
Time

Figure 15. Changes in vascular conductances due to saline treatment in rats with and without aortocaval fistulae.

Volumes equivalent to the largest drug injections were administered to anaesthetized rats every twenty minutes following the control measurement to which subsequent measurements were normalized. All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM. Saline: 67, 200, 67, 200 μL/kg; sham-operated rats: n=10; rats with aortocaval fistulae: n=7. ΔG: change in vascular conductance; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

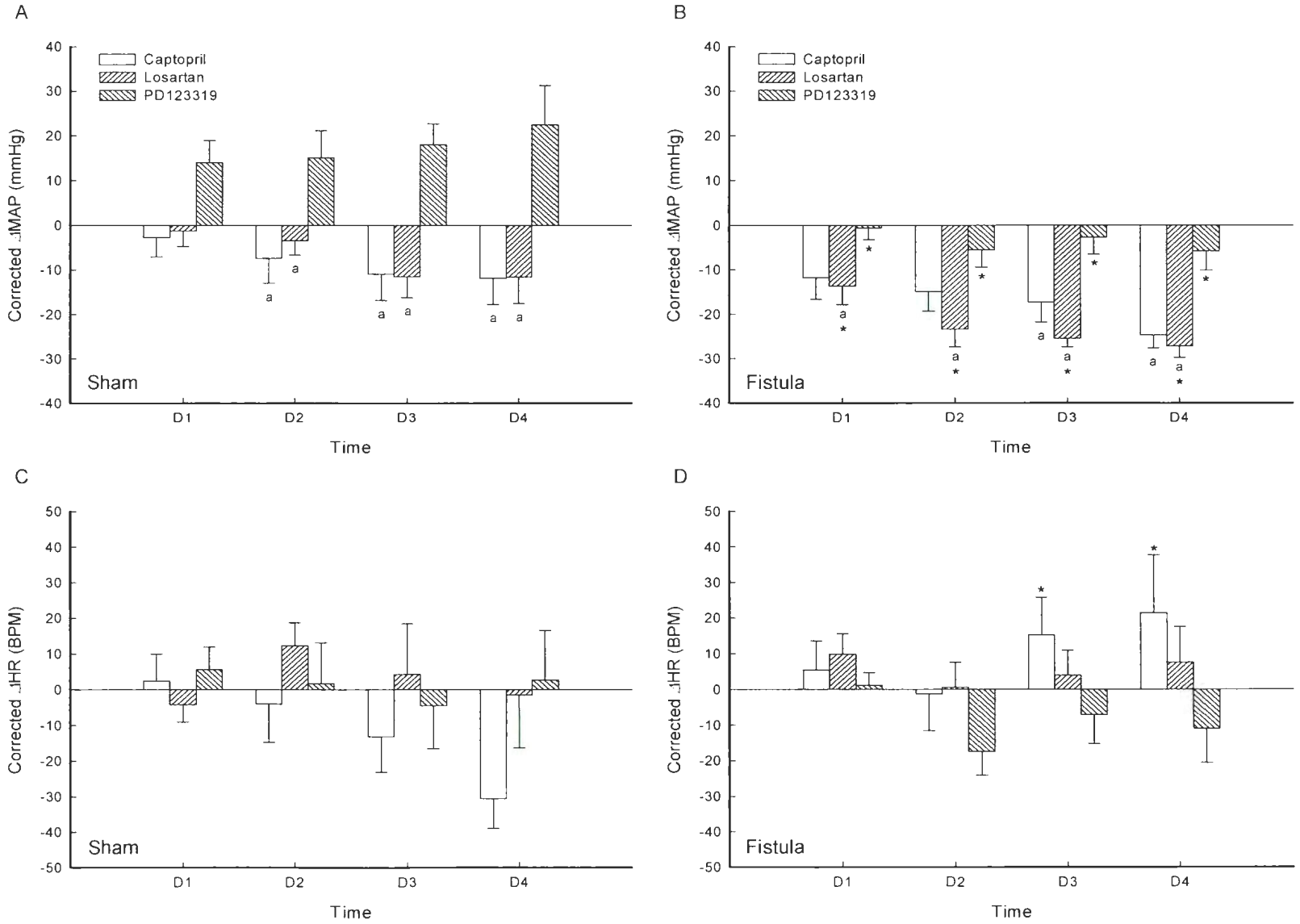
A
B
C
D
Captopril
Losartan
PD123319
Corrected ΔMAP (mmHg)
Corrected ΔHR (BPM)
Sham
Fistula
Time
D1
D2
D3
D4
a
*

Figure 16. Changes in mean arterial pressure and heart rate due to drug treatments in rats with and without aortocaval fistulae. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg; sham-operated rats: n=7 (captopril), 9 (losartan), 8 (PD 123319); rats with aortocaval fistulae: n=6 (captopril), 9 (losartan), 8 (PD 123319). SO: sham-operated; ΔMAP: change in mean arterial pressure; ΔHR: change in heart rate; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

[a]significantly different from the PD 123319 group at the same dose time, $p<0.05$

[*]significantly different from the respective sham value, $p<0.05$

3.8. Comparison of the effects of captopril, losartan, and PD 123319 on aortic blood flow and conductance

In sham-operated rats, the decreases in corrected aortic blood flow (cΔABF) produced by captopril and losartan were similar to the increase in cΔABF produced by PD 123319 (Figure 17A). In rats with aortocaval fistulae, the dose-dependent decrease in cΔABF produced by captopril was significantly greater than that produced by PD 123319 (Figure 17B). The decreases in cΔABF produced by captopril and losartan were significantly greater in rats with aortocaval fistulae than in sham-operated rats. Similarly, the decrease in cΔABF produced by PD 123319 in rats with aortocaval fistulae was significantly different from the increase in cΔABF in sham-operated rats.

In sham-operated rats, the increases in corrected aortic conductance (cΔAC) produced by captopril and losartan were not significantly different from the decrease in cΔAC produced by PD 123319 (Figure 17C). In rats with aortocaval fistulae, the increase in cΔAC produced by losartan was significantly different from the variable effect of PD 123319 on cΔAC (Figure 17D). However, the increase in cΔAC produced by captopril was similar to both the effects of losartan and PD 123319. Captopril and losartan increased cΔAC significantly more in rats with aortocaval fistulae than in sham-operated rats. The decreases produced by PD 123319 were similar in both groups.

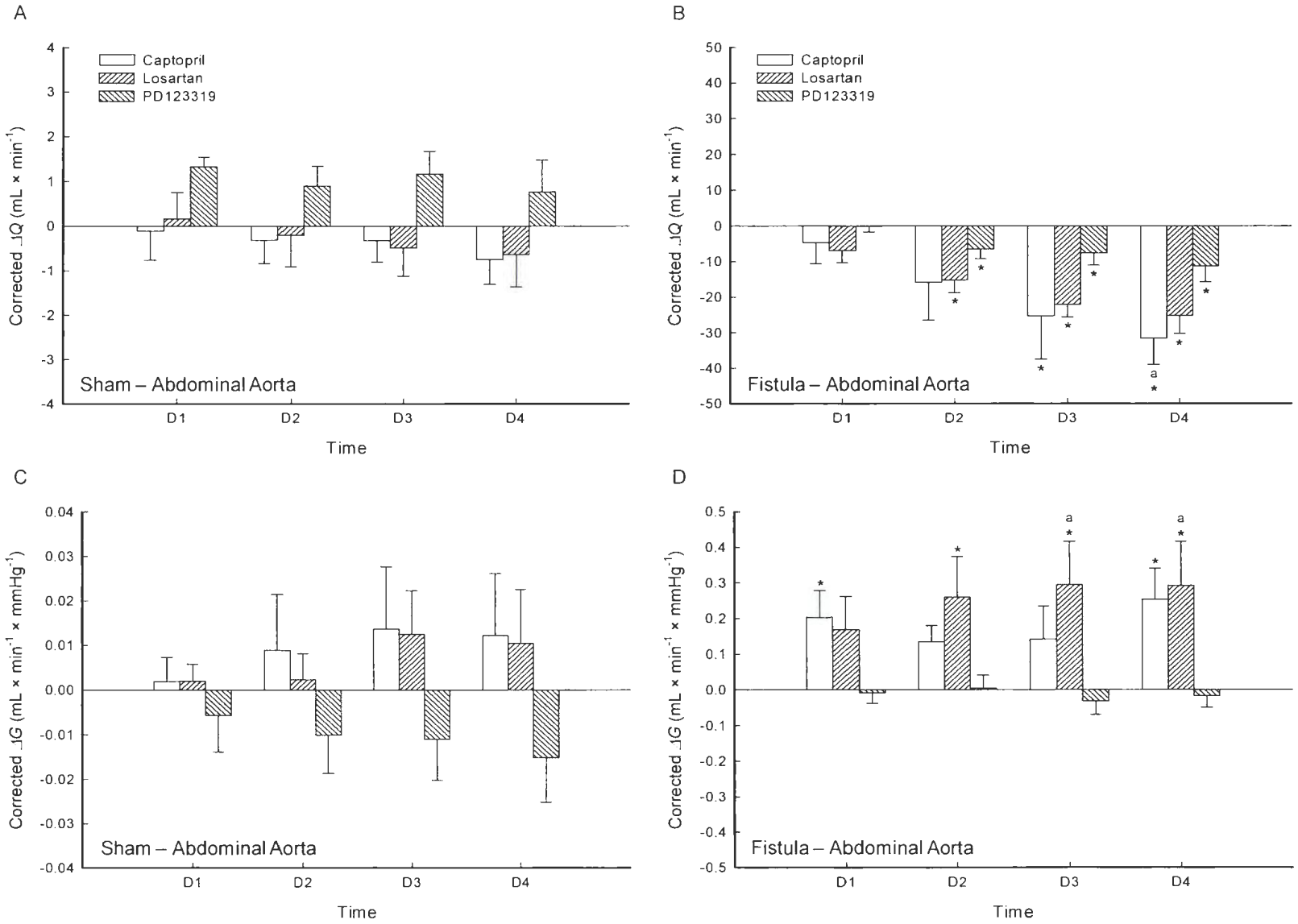
A
Captopril
Losartan
PD123319
Corrected ΔQ (mL × min-1)
Sham – Abdominal Aorta
D1
D2
D3
D4
Time
B
Captopril
Losartan
PD123319
Corrected ΔQ (mL × min-1)
Fistula – Abdominal Aorta
D1
D2
D3
D4
Time
C
Corrected ΔG (mL × min-1 × mmHg-1)
Sham – Abdominal Aorta
D1
D2
D3
D4
Time
D
Corrected ΔG (mL × min-1 × mmHg-1)
Fistula – Abdominal Aorta
D1
D2
D3
D4
Time

Figure 17. Changes in aortic blood flow and conductance due to drug treatments in rats with and without aortocaval fistulae. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg; sham-operated rats: n=7 (captopril), 9 (losartan), 8 (PD 123319); rats with aortocaval fistulae: n=6 (captopril), 9 (losartan), 8 (PD 123319). SO: sham-operated; ΔQ: change in blood flow; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

[a]significantly different from the PD 123319 group at the same dose time, $p<0.05$

[*]significantly different from the respective sham value, $p<0.05$

3.9. Comparison of the effects of captopril, losartan, and PD 123319 on mesenteric blood flow and conductance

Captopril, losartan, and PD 123319 increased corrected mesenteric blood flow (cΔMBF) to similar extents in sham-operated rats (Figure 18A). In rats with aortocaval fistulae, the dose-dependent increase in cΔMBF produced by losartan was significantly different from the decrease in cΔMBF produced by PD 123319 (Figure 18B). Captopril increased cΔMBF in rats with aortocaval fistulae, this was not significantly different from the effects of losartan or PD 123319. The decrease in cΔMBF produced by PD 123319 in rats with aortocaval fistulae was significantly different from the increase in cΔMBF in sham-operated rats. The increases in cΔMBF produced by captopril and losartan were similar between rats with and without aortocaval fistulae.

In sham-operated rats and in those with aortocaval fistulae, the dose-dependent increases in corrected mesenteric conductance (cΔMC) produced by captopril and losartan were significantly different from the decrease in cΔMC produced by PD 123319 (Figure 18CD). The increase in cΔMC produced by losartan was significantly larger in rats with aortocaval fistulae than in sham-operated rats, while the effects of captopril and PD 123319 were similar in both groups.

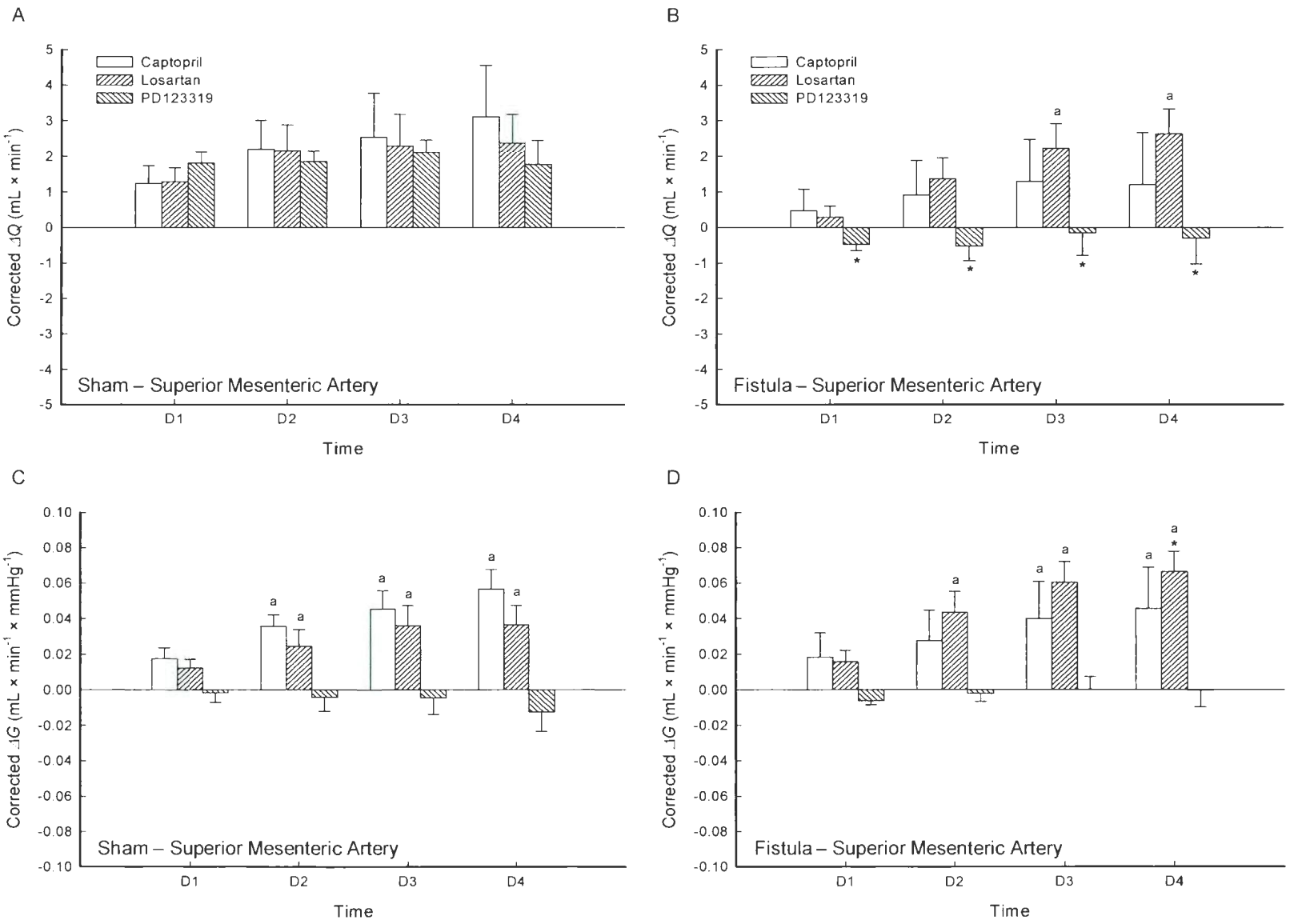
A
Captopril
Losartan
PD123319
Corrected ΔQ (mL × min-1)
Sham – Superior Mesenteric Artery
D1
D2
D3
D4
Time
B
Captopril
Losartan
PD123319
Corrected ΔQ (mL × min-1)
Fistula – Superior Mesenteric Artery
D1
D2
D3
D4
Time
C
Corrected ΔG (mL × min-1 × mmHg-1)
Sham – Superior Mesenteric Artery
D1
D2
D3
D4
Time
D
Corrected ΔG (mL × min-1 × mmHg-1)
Fistula – Superior Mesenteric Artery
D1
D2
D3
D4
Time

Figure 18. Changes in mesenteric blood flow and conductance due to drug treatments in rats with and without aortocaval fistulae. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg; sham-operated rats: n=7 (captopril), 9 (losartan), 8 (PD 123319); rats with aortocaval fistulae: n=6 (captopril), 9 (losartan), 8 (PD 123319). SO: sham-operated; ΔG: change in vascular conductance; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

[a]significantly different from the PD 123319 group at the same dose time, $p<0.05$

[*]significantly different from the respective sham value, $p<0.05$

3.10. Comparison of the effects of captopril, losartan, and PD 123319 on renal blood flow and conductance

In sham-operated rats, captopril and losartan had variable effects on corrected renal blood flow (cΔRBF) that were not significantly different from the increase in cΔRBF produced by PD 123319 (Figure 19A). In contrast, the increase in cΔRBF produced by losartan was significantly different from the decreases in cΔRBF produced by captopril and PD 123319 in rats with aortocaval fistulae (Figure 19B). The decrease in cΔRBF produced by PD 123319 and the increase produced by losartan in rats with aortocaval fistulae were significantly different from the variable effects of losartan and the increase produced by PD 123319 in sham-operated rats.

In sham-operated rats, the increases in corrected renal conductance (cΔRC) produced by captopril and losartan were significantly different from the decrease in cΔRC produced by PD 123319 (Figure 19C). However, the increase in cΔRC produced by losartan in rats with aortocaval fistulae was significantly different from the smaller increase in cΔRC produced by captopril and the decrease in cΔRC caused by PD 123319 (Figure 19D). The decrease in cΔRC produced by PD 123319 in rats with aortocaval fistulae was significantly smaller than the decrease in cΔRC produced by PD 123319 in sham-operated rats. In contrast, the increases in cΔRC produced by captopril and losartan were similar between rats with and without aortocaval fistulae.

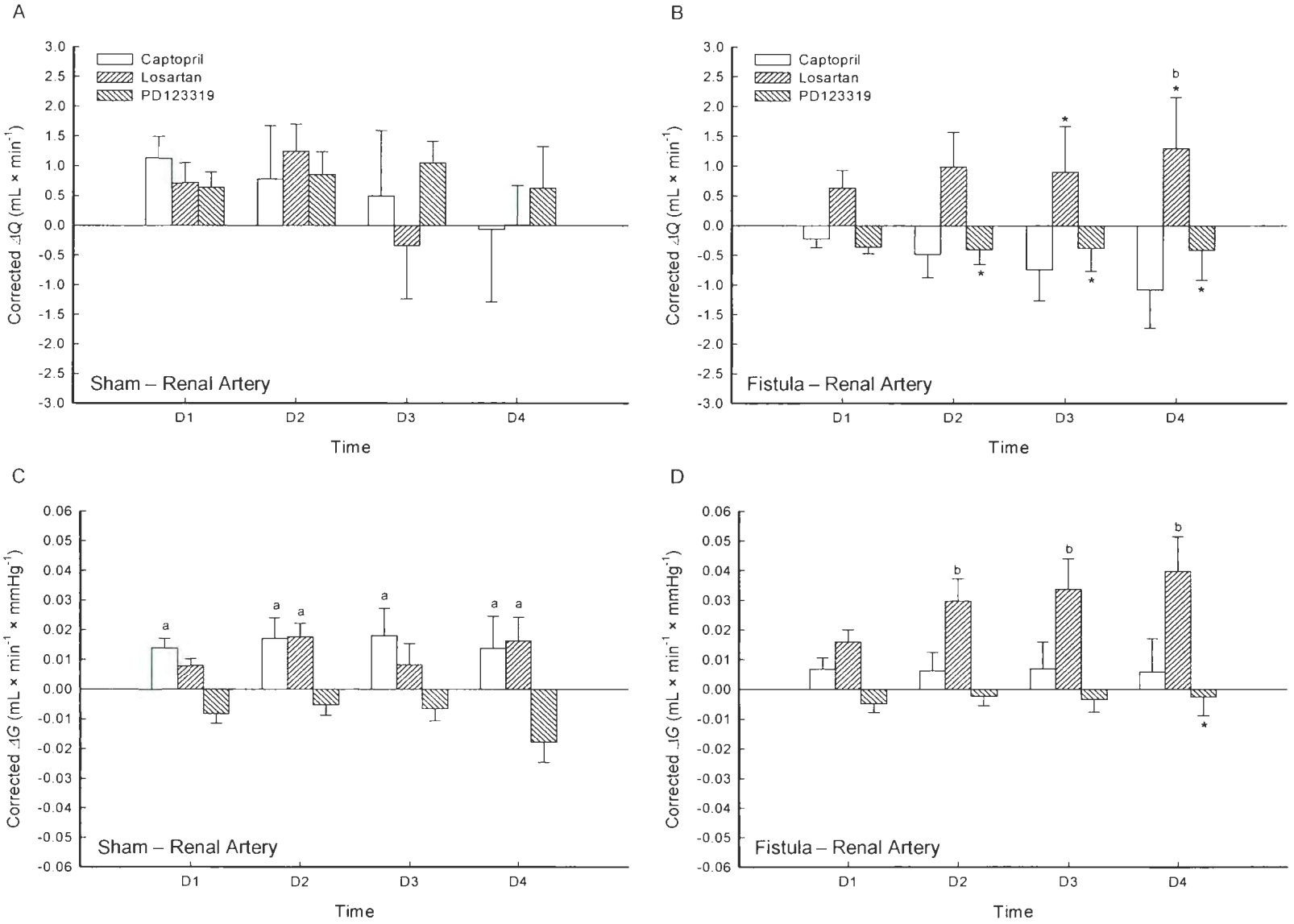
A
B
C
D
Captopril
Losartan
PD123319
Corrected ΔQ (mL × min-1)
Corrected ΔG (mL × min-1 × mmHg-1)
Sham – Renal Artery
Fistula – Renal Artery
Time
D1
D2
D3
D4

Figure 19. Changes in renal blood flow and conductance due to drug treatments in rats with and without aortocaval fistulae. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg; sham-operated rats: n=7 (captopril), 9 (losartan), 8 (PD 123319); rats with aortocaval fistulae: n=6 (captopril), 9 (losartan), 8 (PD 123319). SO: sham-operated; ΔQ: change in blood flow; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

[a]significantly different from the PD 123319 group at the same dose time, $p<0.05$

[b]significantly different from the captopril and PD 123319 groups at the same dose time, $p<0.05$

[*]significantly different from the respective sham value, $p<0.05$

3.11. Comparison of the effects of captopril, losartan, and PD 123319 on iliac blood flow and conductance

The variable effects of captopril and PD 123319 on corrected iliac blood flow (cΔIBF) were not significantly different from the decrease in cΔIBF produced by losartan in sham-operated rats (Figure 20A). Similarly, the decreases in cΔIBF produced by captopril, losartan, and PD 123319 were similar in rats with aortocaval fistulae (Figure 20B). Furthermore, the effects of captopril, losartan, and PD 123319 were similar between rats with and without aortocaval fistulae.

In sham-operated rats, the increase in corrected iliac conductance (cΔIC) produced by captopril was significantly different from the dose-dependent decrease in cΔIC produced by PD 123319 (Figure 20C). Similarly, the increase in cΔIC produced by losartan was significantly different from the decrease in cΔIC produced by PD 123319 in rats with aortocaval fistulae (Figure 20D). The effects of captopril, losartan, and PD 123319 were similar between rats with and without aortocaval fistulae.

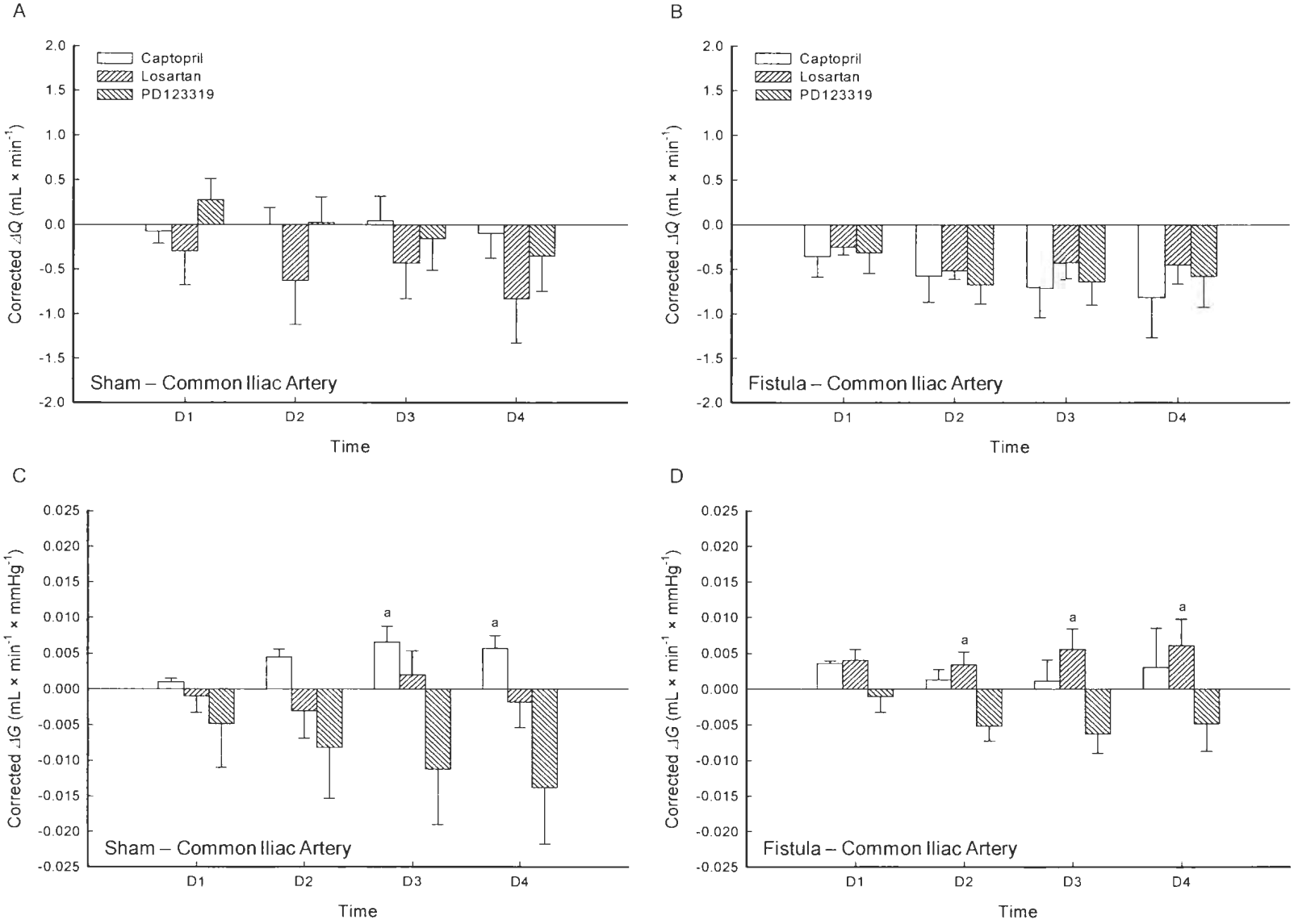
A
B
C
D
Captopril
Losartan
PD123319
Corrected ΔQ (mL × min-1)
Corrected ΔG (mL × min-1 × mmHg-1)
Sham – Common Iliac Artery
Fistula – Common Iliac Artery
D1
D2
D3
D4
Time
a

Figure 20. Changes in iliac blood flow and conductance due to drug treatments in rats with and without aortocaval fistulae. Doses were administered every twenty minutes following the control measurement to which subsequent measurements were normalized. All conductances were calculated using mean arterial pressure measured in the external iliac artery. Values are mean ± SEM. Captopril: 0.3, 1.0, 3.0, 10.0 mg/kg; losartan: 2.5, 5.0, 10.0, 20.0 mg/kg; PD 123319: 0.1, 0.3, 1.0, 3.0 mg/kg; sham-operated rats: n=7 (captopril), 9 (losartan), 8 (PD 123319); rats with aortocaval fistulae: n=6 (captopril), 9 (losartan), 8 (PD 123319). SO: sham-operated; ΔQ: change in blood flow; D1: dose 1; D2: dose 2; D3: dose 3; D4: dose 4.

[a]significantly different from the PD 123319 group at the same dose time, $p<0.05$

4. Discussion

We hypothesized that the RAAS has a greater influence over blood flow distribution in rats with aortocaval fistulae than in sham-operated rats. To test this hypothesis, changes in regional blood flows and conductances produced by captopril, losartan, and PD 123319 were compared between these two groups of rats. Overall, the results of this study suggest that Ang II is more active in the continuous control of regional haemodynamics in rats with aortocaval fistulae than in sham-operated rats.

We also hypothesized that the increases in regional blood flows and conductances produced by AT_1 receptor antagonism would be larger than the decreases produced by AT_2 receptor antagonism, in rats with and without aortocaval fistulae. To test this hypothesis, changes in regional blood flows and conductances produced by losartan and PD 123319 were compared within each group of rats. The results suggest that AT_1 receptors are more active than AT_2 receptors in the continuous control of regional haemodynamics in rats with aortocaval fistulae. In contrast, the continuous control of regional haemodynamics by AT_1 and AT_2 receptors in sham-operated rats is regionally specific.

The results of this study suggest that the activated RAAS of rats with aortocaval fistulae has a greater influence in controlling regional haemodynamics than in sham-operated rats. Furthermore, the RAAS is altered in rats with aortocaval fistulae to favor vasoconstriction via AT_1 receptors, possibly as a mechanism of maintaining a normal mean arterial pressure despite a large decrease in total peripheral resistance. Although the experimental technique of this study cannot elucidate changes in AT_1 and AT_2 receptor

expression, the introduction of aortocaval fistulae likely causes a molecular change at the receptor level. For example, vasoconstriction might be favored in rats with aortocaval fistulae by upregulation of AT_1 receptors and/or downregulation of AT_2 receptors.

4.1. Ang II control of regional haemodynamics in rats with and without aortocaval fistulae

Captopril caused similar changes in mean arterial pressure, regional blood flows and regional conductances in rats with and without aortocaval fistulae (Figures 15B, 17BD, 18BD, and 19BD). This suggests that the continuous control of regional haemodynamics is similar between the two groups. However, the effects of activated AT_1 and AT_2 receptors generally oppose each other, and the dual attenuation of AT_1 and AT_2 receptor activation by ACE inhibitors could result in unchanged regional haemodynamics (review: Nguyen Dinh Cat and Touyz, 2011).

This study has shown that AT_1 receptors exert a greater influence than AT_2 receptors over regional haemodynamics in rats with aortocaval fistulae (Figures 17BD, 18BD, and 19D). In comparison, both receptors exert variable influence over regional haemodynamics in sham-operated rats (Figures 17C, 18C, and 19C). As such, ACE inhibition would be expected to result in vasodilation in rats with aortocaval fistulae by attenuation of AT_1 receptor activation. Similarly, attenuation of AT_1 receptor activation should cause vasodilation in sham-operated rats. However, the added attenuation of AT_2 receptor activation in these rats would negate some of the vasodilation produced by attenuation of AT_1 receptor activation. Therefore, the changes in regional conductances

of rats with aortocaval fistulae was expected to be significantly larger than in sham-operated rats.

This hypothesis is inconsistent with the results that when treated with captopril the changes in all regional conductances were greater, although not significantly greater, in sham-operated rats than in rats with aortocaval fistulae (Figures 17D, 18D, and 19D). However, AT_1 receptor antagonism did produce the expected result of larger changes in all regional conductances in rats with aortocaval fistulae than in sham-operated rats, although this was only significant in the mesenteric bed (Figures 17D, 18D, and 19D). The upregulation of enzymes capable of ACE-independent Ang II production throughout the vasculature or sufficiently within a single region to produce systemic effects could explain the inability of captopril to modify regional conductance in rats with aortocaval fistulae. Enzymes capable of ACE-independent Ang II production are present in rat vascular beds and tissues; they appear to by upregulated in some rat models of hypertension (Santos et al., 2003; Guo et al., 2001).

Administration of losartan to rats with aortocaval fistulae resulted in a significantly larger decrease in mean arterial pressure, and significantly larger increases in mesenteric conductance and renal blood flow, than in sham-operated rats (Figures 15B, 17D, and 18B). This suggests that Ang II activation of AT_1 receptors exerts greater control over regional haemodynamics in rats with aortocaval fistulae than in sham-operated rats.

In sham-operated rats, PD 123319 significantly increased mean arterial pressure, mesenteric blood flow, and renal blood flow, and significantly decreased renal

conductance, compared to rats with aortocaval fistulae (Figures 15B, 17B, and 18BD). This suggests that Ang II activation of AT_2 receptors exerts greater control over regional haemodynamics in sham-operated rats than in rats with aortocaval fistulae.

4.2. AT_1 and AT_2 receptor control of regional haemodynamics in rats with aortocaval fistulae

In rats with aortocaval fistulae, losartan decreased mean arterial pressure more than PD 123319. Furthermore, losartan increased mesenteric, renal, and iliac conductances, whereas PD 123319 caused small decreases in these conductances (Figures 15B, 16D, 18D, and 19D). These results suggest that AT_1 receptors exert a greater influence than AT_2 receptors in the control of haemodynamics in rats with aortocaval fistulae. Given the smaller effects of PD 123319 on mean arterial pressure and regional conductances, it would seem that Ang II activation of AT_2 receptors exerts little influence over regional haemodynamics in rats with aortocaval fistulae.

However, losartan increased renal conductance significantly more than captopril, yet captopril and losartan yielded similar effects on mean arterial pressure and mesenteric conductance (Figures 18D, 15B, and 17B). The observed lack of captopril-mediated alteration in renal conductance might be explained on a tissue rather than systemic level and seems to be related to the presence of aortocaval fistulae, as losartan and captopril increased renal conductance similarly in sham-operated rats (Figure 19C).

The attenuated effect of captopril on renal conductance in rats with aortocaval fistulae might indicate regionally specific upregulation of ACE-independent Ang II

production in rats with aortocaval fistulae (Figure 19D). Others have found increased ACE-independent Ang II production in the ischaemic kidneys of two-kidney one-clip rats and dogs (Sadjadi et al., 2005; Tokuyama et al., 2002). The ACE-independent pathways of Ang II production were attributed to chymase or chymase-like activity, potentially that of elastase-2 (Santos et al., 2003; Guo et al., 2001). Rats with aortocaval fistulae have a reduced renal blood flow, this specifically affects the renal cortex (Abassi et al., 1998a). If the renal ischaemia produced in rats with aortocaval fistulae results in renal upregulation of ACE-independent Ang II production, it would explain the attenuated renal effect of captopril compared to losartan.

Alternatively, it is possible that antagonism of AT_2 receptors is trivial compared to the effects of activated AT_1 receptors. However, in the setting of AT_1 receptor antagonism, the AT_2 receptors may play a large role in the observed renal vasodilation (Figure 19D). In this case, ACE inhibition would dually attenuate the activation of AT_1 and AT_2 receptors, and the vasodilatory actions of activated AT_2 receptors would be null. Regardless, ACE inhibition may favor vasodilation by mechanisms other than attenuation of AT_1 receptor activation.

ACE inhibition by captopril should simultaneously attenuate ACE-mediated bradykinin degradation and may result in increased Ang 1-9 production by ACE2 produced by decreased substrate competition (review: Nguyen Dinh Cat and Touyz, 2011; Kurdi et al., 2005). Ang 1-9 has been observed to potentiate bradykinin B_2 receptor release of NO, and therefore may contribute to captopril-mediated vasodilation (Jackman et al., 2002). Finally, neprilysin can convert Ang I to Ang 1-7 (review: Ferrario et al.,

1998). Ang 1-7 activation of Mas and AT_2 receptors would result in further vasodilation during ACE inhibition by captopril (Ocaranza et al., 2010; review: Rabelo et al., 2011).

4.3. AT_1 and AT_2 receptor control of regional haemodynamics in sham-operated rats

The increase in mean arterial pressure and decreases in mesenteric conductance and renal conductance produced by losartan, along with the opposing effects of PD 123319 on these parameters, indicates that AT_1 and AT_2 receptors are continuously active in sham-operated rats (Figures 15A, 17C, and 18C). Yet, there appears to be no haemodynamic differences between captopril and losartan administration in sham-operated rats (Figures 15A, 17AC, 18AC, and 19AC). With ACE inhibition by captopril, the attenuation of AT_2 receptor activation, which should cause vasoconstriction, might be overshadowed by other vasodilatory mechanisms during ACE inhibition.

The comparable decreases in mean arterial pressure, and increases in renal and mesenteric conductance, produced by captopril and losartan administration would seem to suggest that all Ang II effects are mediated by activation of AT_1 receptors in sham-operated rats (Figures 15A, 17C, and 18C). However, AT_2 receptor antagonism increased mean arterial pressure and decreased mesenteric, renal, and iliac conductances (Figures 15A, 17C, 18C, and 19C). It appears that ACE inhibition produces functionally different effects than the sum of individual AT_1 and AT_2 receptor antagonism. Again, this may be explained by other vasodilatory mechanisms as consequences of ACE inhibition.

Although drug treatments produced no change in blood flows, vascular conductances varied with drug treatment. Captopril and losartan increased mesenteric and

renal conductances, while PD 123319 decreased renal and hindquarter conductances (Figure 9BCD). These observations suggest that the mesenteric and renal vascular tones are continually controlled by activation of AT_1 receptors in sham-operated rats. In contrast, AT_2 receptor antagonism resulted in significant renal and hindquarter vasoconstriction. This suggests maintenance of renal and hindquarter vascular conductances by Ang II activation of AT_2 receptors in sham-operated rats. However, it is particularly possible that the decrease in renal conductance was not a direct consequence of renal AT_2 receptor antagonism, but due to auto-regulatory responses to maintain normal blood flow despite increased mean arterial pressure produced by PD 123319 antagonism of AT_2 receptors (Hill et al., 2010).

It is perhaps surprising that captopril and losartan did not significantly decrease mean arterial pressure in sham-operated rats. This is likely because the decrease in mean arterial pressure was compared to the sizeable decrease produced by normal saline, and because the RAAS is not overtly activated in sham-operated rats (Figure 7A). Similarly, Gonzalez-Perez et al. (1985) observed no significant change in femoral pressure when comparing the decrease produced by captopril (5 mg/kg) to normal saline injected rats.

4.4. Ventricular hypertrophy in rats with aortocaval fistulae

Similar to other studies, rats with aortocaval fistulae developed left and right ventricular hypertrophy (Guo and Tabrizchi, 2008; Willenbrock et al., 1999b). The ratio of left to right ventricular weight was similar between the groups of rats (Table 3). This suggests that the left and right ventricles hypertrophied to similar extents. Liu et al.

(1991) measured increased left and right ventricular end diastolic pressures in rats with aortocaval fistulae. This might account for the elevated left ventricular end diastolic wall stress of rats with aortocaval fistulae (Gladden et al., 2011; Hutchinson et al., 2011). In volume overload, increased ventricular wall thickness and increased ventricular chamber size occur with increased left ventricular wall stress (Grossman et al., 1975). Enlargement of the ventricular chambers and thickening of ventricular walls reduces wall stress (Liu et al., 1991). Therefore, this study suggests that right ventricular wall stress is also increased, and that the left and right ventricles hypertrophied to similar extents to mitigate increased wall stress.

4.5. The haemodynamic stability of rats with and without aortocaval fistulae

The haemodynamic stability of rats with and without aortocaval fistulae differed with respect to mean arterial pressure and mesenteric blood flow, as assessed by normal saline injection (Figures 12A and 13B). Generally, rats with aortocaval fistulae maintained both parameters at or above their baseline values, whereas time-dependent decreases in both parameters were observed in sham-operated rats.

Guo and Tabrizchi (2008) observed no decrease in mean arterial pressure of sham-operated rats infused with normal saline. Given the similar preparation of rats with aortocaval fistulae, the different trends might be produced by experimental protocols. Guo and Tabrizchi (2008) inserted catheters into both iliac veins and arteries, as well as into the left ventricle and a balloon-tipped catheter into the right atrium. The additional instrumentation or the recurrent cessation of blood flow for measurement of mean

circulatory filling pressure might have resulted in activation of sympathetic responses that maintained mean arterial pressure.

The presence of aortocaval fistulae may explain the ability of rats with aortocaval fistulae to maintain mean arterial pressure and mesenteric blood flow. In rats with aortocaval fistulae, Guo and Tabrizchi (2008) increased mean arterial pressure using noradrenaline and vasopressin. However, the mean arterial pressure of these rats was not decreased by sodium nitroprusside. Cardiac index and arterial resistance remained unchanged with all treatments. Aortocaval fistulae have a substantial influence over total peripheral resistance produced by the large volume of blood shunted from the aorta into the inferior vena cava. Similarly, as the fistula has a large conductance it has great influence over venous return and therefore cardiac output. Given that cardiac index and total peripheral resistance are seemingly invariable in rats with aortocaval fistula, a more stable mean arterial pressure is expected than in sham-operated rats.

4.6. Baseline haemodynamic values of rats with and without aortocaval fistulae

In this study, the mean arterial pressures of rats with and without aortocaval fistulae were slightly lower and the heart rates generally higher than those of age matched studies (Guo and Tabrizchi, 2008; Huang et al., 1992). The different experimental protocols (e.g. anesthetics, fistula sizes) may explain the variable findings. Regardless, all studies have concluded that rats with and without aortocaval fistulae have similar mean arterial pressures and heart rates (Figure 5AB).

Mesenteric and renal blood flows and conductances were reduced in rats with aortocaval fistulae compared to sham operated rats, whereas the iliac blood flows and conductances were similar (Figure 6BD). Reduced renal blood flow had been previously measured by microspheres at five weeks after fistula induction (Huang et al., 1992). In rats with aortocaval fistulae, total peripheral resistance is decreased, although increased cardiac output maintains a normal mean arterial pressure (Huang et al., 1992). Systemic vasoconstriction likely occurs as a mechanism to increase total peripheral resistance and aid in maintaining a normal mean arterial pressure. As the renal and mesenteric vascular beds have larger blood flows than the hindquarters, and therefore greater propensities to influence total peripheral resistance, it may be reasonable to assume that they would constrict while the hindquarter conductance might remain unchanged.

4.7. Sources of error

In sham-operated rats, the mean mesenteric blood flows of the losartan and PD 123319 groups were significantly lower than those of the captopril and normal saline groups. Furthermore, the mean mesenteric conductance of the losartan group was significantly lower than those of the captopril and normal saline groups. This source of error was carried throughout the analysis of drug effects in sham-operated rats and in their comparison to rats with aortocaval fistulae.

Also, the use of captopril in this study highlighted the incomplete attenuation of the RAAS by ACE inhibition in rats with aortocaval fistulae. As the captopril group was intended to provide an overall assessment of the integrated AT_1 and AT_2 receptor

influence on regional haemodynamics, it may not have been accurate to compare the overall influence of Ang II in rats with and without aortocaval fistulae based on data obtained by captopril inhibition of ACE.

4.8. Future studies

It would be interesting to test whether the simultaneous effects of losartan and PD 123319 are comparable to those of captopril in rats with and without aortocaval fistulae. Furthermore, it would be interesting to assay for ACE-independent Ang II production at the cellular level within the vascular and perivascular tissues of rats with aortocaval fistulae. In addition, dose response curves to losartan following pretreatment with PD 123319, and vice versa, might help to more accurately indicate the level of control that each receptor subtype exerts over regional haemodynamics in rats with and without aortocaval fistulae. Finally, quantitatively examining AT_1 and AT_2 receptor distribution might provide additional information regarding the mechanism of altered Ang II control of regional haemodynamics in rats with aortocaval fistulae.

4.9. Conclusion

Ang II is more active in the continuous control regional haemodynamics in rats with aortocaval fistulae than in sham-operated rats. In rats with aortocaval fistulae, AT_1 receptors are more active than AT_2 receptors in regulating regional blood flows and conductances, whereas the input from AT_1 and AT_2 receptors in sham-operated rats is regionally specific. These findings suggest that the RAAS is altered in rats with

aortocaval fistulae to favor vasoconstriction, possibly as a mechanism of maintaining a normal mean arterial pressure despite a large decrease in total peripheral resistance.

Rats with aortocaval fistulae are a model of decreased systemic blood flow. The results of this study suggest that AT_1 receptor antagonism is more effective at increasing regional blood flow than ACE inhibition, in rats with aortocaval fistulae. This may be true of other pathological conditions in which tissue ischaemia results from systemic vasoconstriction and decreased systemic blood flow.

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